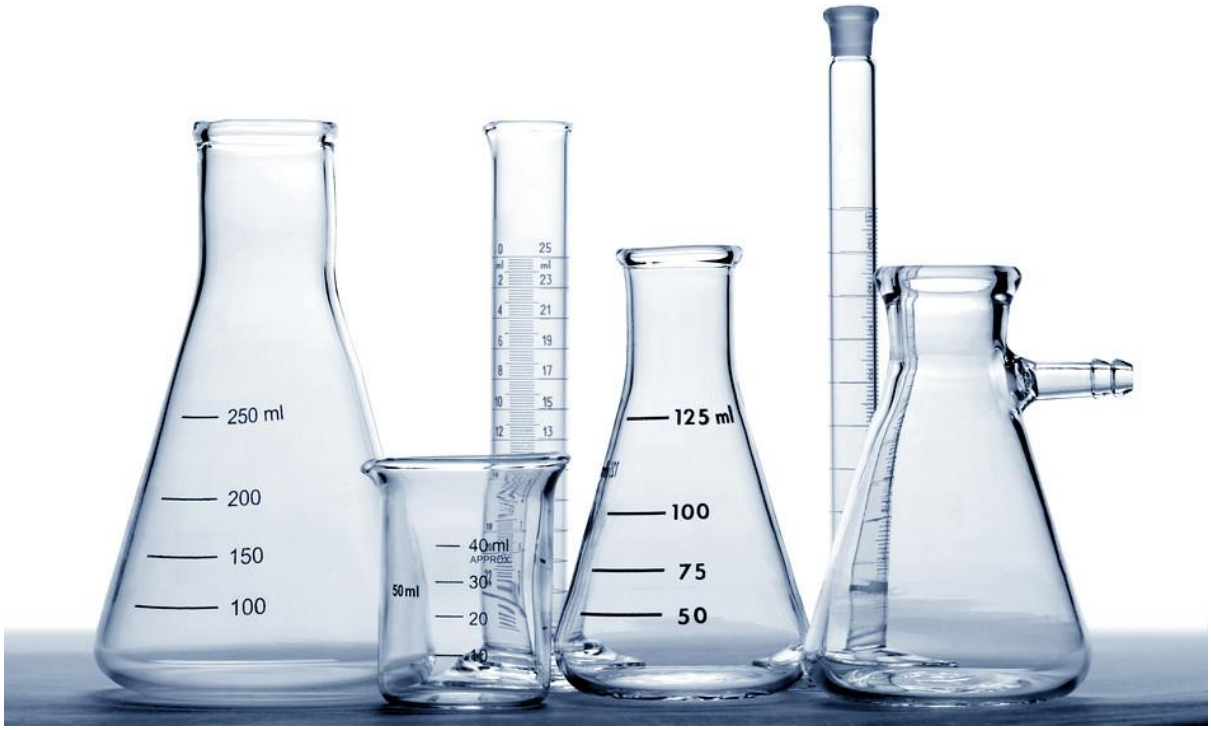
LABORATORY MANUAL



FAHIM SEAFOOD PROCESSING & FARMING LIMITED

Edition: 1st, 2023

Factory Address:

Debitala, Batiaghata, Khulna, Bangladesh

Phone: +880 1970 333 267

Email: fahimsfpf@gmail.com

Approved By

Managing Director

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COMMITMENT

Microbiologic test principles, a scientific method, which provides best control over the safety of foods products for human. The management of the company laboratory honestly desire to follow and implement the Microbiological test principles in the laboratory with their best effort and the confident to laboratory test, which is ensured to produce safety foods products according to the rules and regulation described in the microbiological analysis manual.

Approved By

Managing Director

AMENDMENT SHEET

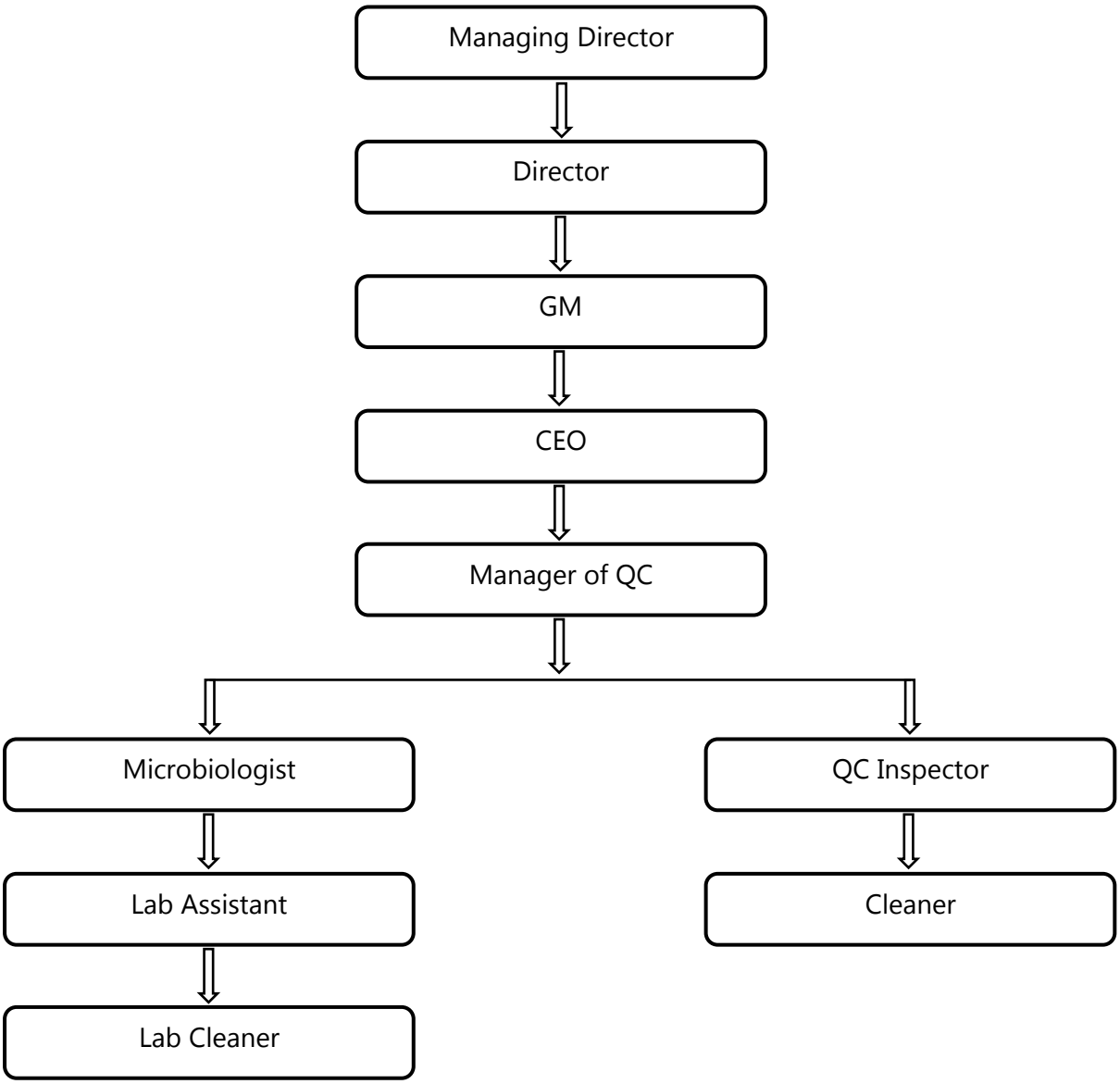
The First edition of Laboratory Manual is updated one. Latest scientific information, Ideas and experiences have been in this edition for test/ analysis procedure.

Date	Subject	Page No.	Signature of Manger, QC

Approved By

Managing Director

ORGANOGRAM OF QC DEPARTMENT



Approved By

Managing Director

ORGANIZATION CHART NARRATIVE

MANAGING DIRECTOR:

Fahim Seafood Processing & Farming Limited is a Private Limited Company and the Managing Director is the Chief Executive of the company who has got sound knowledge and vast experience in this trade. He formulates policy in consultation with other executives regarding operations of the factory in conformity with day to day changing and advancement of modern food technology. He reviews the overall performance of the factory form purchasing materials to sales and implementation of HACCP system.

DIRECTOR:

Director is the key person of the project. He executes all official function including administration, production as well as quality assurance program. He is only liable to board of directors for his works and activities. He is empowered to implement the microbiological practices.

GENERAL MANAGER:

General Manager look after the activities related to administration, finance, procurement, production and quality control. He also maintains liaison with all organization concerned with the trade. He is responsible to the Managing Director for the implementation and complying with HACCP principles.

CHIEF EXECUTIVE OFFICER:

Chief Executive Officer look after the activities related to administration, finance, procurement, production and quality control. He also maintains liaison with all organizations concerned with the trade. He is responsible to the managing director for the implementation and complying with HACCP principles. He is also responsible for implementing and complying with HACCP regulation in our processing plant. He is also responsible to maintain all necessary works in general administration, construction and mechanical activities, production, procurement and marketing. He is directly responsible to the director for all his activity.

MANAGER OF QUALITY CONTROL:

The key person of the HACCP as the Quality Assurance, who is solely responsible HACCP and Laboratory rules and regulation and to maintain the safety and quality foods product. He is responsible for reviewing, monitoring and corrective activities. He coordinates with all the Laboratory persons. He is liable to the Managing Director & Director beside other senior' s counter-part.

QC INSPECTOR:

He is responsible for monitoring activities like cleaning, sanitizing and hygiene practices in the factory and laboratory. He is liable to Manager of Quality Control.

MEDICAL OFFICER:

He is responsible for maintaining medical documents and workers health. He is liable to the General Manager.

MICROBIOLOGIST:

He is the Laboratory In-charge and works microbiological aspects of the process line and production monitoring. Being a member of the HACCP team he is liable to Manager of Quality Control.

LAB-ASSISTANT:

He is the laboratory assistant and works with the microbiological aspects of the process line and production monitoring. He is liable the microbiologist.

LAB-CLEANER:

He is the laboratory cleaner and works with Lab assistant and microbiologist. He cleans the all equipment and instrument of the laboratory. He is liable to the laboratory cleaning side.

DESCRIPTION OF MICROBIOLOGICAL LABORATORY FACILITY

A Laboratory should be designed and constriction such that the most important part of the facilities is the safety of the workers that used it. The foods microbiological laboratory should be properly equipped to carry out the stated objectives.

a) A sample space for the safe conduct of laboratory work and for cleaning and maintained.
b) Wall, ceiling and floors are smooth, each to clean, impermeable to liquid and resistant to the chemical and disinfectants normally used in the laboratory. Floors are resistant.
c) The Laboratory is well ventilated. Normally, an ambient temperature of 21 °C to 25 °C and relative humidity of 45 percent to 50 percent is recommended.
d) Laboratory lighting are maintained at average intensity of 50 to 100 feet candles direct sunlight is known to have adverse effects on media, reagent and some type of specimens.
e) Laboratory space and bench areas shall, for most routine work, be six linear feet as the recommended minimum work area shall for each analysis. The idea bench top height is 35 inches with depth of 35 to 40 inches.
f) Bench top is made of SS, Bench top is impervious to water and resistant to disinfectant, acids, alkalis organic solvents and moderate heat.
g) Storage space for equipment, materials and samples are sufficient for needed media, reagents, glassware and plastic-ware.
h) Facilities for storing outer garments and personal items are provided outside the laboratory working areas.
i) Facilities for eating and drinking and for rest are provided out side the laboratory working areas.
j) Hand washing basins, with running water, if possible, provided in each laboratory room, preferably near the exit door.
k) Door should have vision panels, appropriate fire ratings, and preferable be self closing.
l) There is a reliable and adequate electricity supply and emergency lighting to permit safe exit. A stand-by generator is desirable for support of essential equipment, such as incubator, biological safety cabinets, freezer etc.
m) The laboratory equipped with fire extinguisher system, eyewash station and safety showers.
n) There are in place a documented on-going comprehensive safety education program, which includes the actual operation of safety equipment' s in place in the laboratory.
o) This program shall be documented and monitors for its effectiveness.

The company is completely equipped with a modern microbiological and chemical laboratory for developing the microbiological analysis and company' s own check and self certification system. A full-time microbiologist is in-charge of the laboratory with on lab technician and one lab assistant work. Laboratory people work the third process line with production people and then try to help in realization of microbiological status of products in various stapes as per the national and international. the Microbiologist works with the HACCP team to

developed safety foods in teams of process line and practices. The lab has already required machineries like:

1. Incubator
2. Sterilizer
3. Autoclave
4. Electronic Blender
5. Normal water bath
6. Colony counter
7. pH meter
8. Distilled water plant
9. Balance
10. Microscope

And available glassware. The entire laboratory person is experienced in their duties for long times. Test performed in the laboratory area:

1. Standard plate count (SPC/APC)
2. Total coliform
3. Facal coliform
4. Salmonella
5. Vibrio cholera

Laboratory people performed not only shrimp analysis, but they also swab of food contact surface and hand of staffs of production section, water and ice quality.

EQUIPMENT & QUANTITY

SL NO	Equipment Name	Quantity
01	Incubator	01
02	Sterilizer	01
03	Autoclave	01
04	Colony counter	01
05	Normal water bath	01
06	Circulating water bath	01
07	Electronics Blender	01
08	Distilled water plant	01
09	Temperature meter	01
10	pH meter	01
11	Microscope	01

DESCRIPTION OF EQUIPMENT AND MAINTENANCE

INCUBATOR:

An Incubator consists of a chamber which enables a temperature to within ±1 °C

a. If the ambient temperature is close to or higher than that of the of the incubator, it is necessary to fit a cooling system to the chamber.
b. Petri dishes or tube be placed within 25 mm of the inside wall & separated by at least 25 mm.

MAINTENANCE AND HANDLING:

a. The homogenous temperature shall be checked using thermometer or thermocouples.
b. Always clean of inside/outside.
c. The temperature stability checked by minimum & maximum thermometer.

STERILIZER:

Sterilizer used for dry heat sterilization shall be at 180 °C temperature to destroy the microbes. The sterilizer shall be equipped with-

a. A Thermometer
b. A Thermostat
c. A duration incubator timer.

Volumetric glass ware shall not be sterilized in it. Homogenous temperature shall be maintained. Cleaning, monitoring, Disinfection regularly.

AUTOCLAVE

It is an application which enables a saturated steam temperature at least 121 °C to be attained with a view to destruct the microbes by moist heat. It should be equipped with, at least one safety valve, a pressure gauge, a drain cock, a thermometer and also equipped with a timer. With steam sterilization all air must be expelled, prior to the pressure built up. If the autoclave is not fitted with an automatic evacuation device, it is necessary to remove the air until a continuous jet of steam is emitted.

MAINTENANCE AND HANDLING:

a. Manufactures instruction
b. Monitoring instruction shall be kept in perfect working order
c. Verified decaling & draining regularly
d. Autoclave taps or biological indicator such as ***Bacilus stearothermophilus*** shall be used for proper functioning of the autoclave.

ELECTRONIC BLENDER:

It is used for proper initial suspension from the test sample non liquid products.
This type of apparatus shall not be used which have risk punctuation the bags should be followed Manufactures instruction.

BALANCE:

a. Accuracy should be ±0.01 g.
b. Used for weighing test potion of sample and components of culture media & reagent.

MAINTENANCE AND HANDLING:

a. Placed on a stable horizontal support & also protected from vibration
b. It should be calibrated every working day
c. Entire mechanism should be checked at least once in a year

pH METER:

It should be capable of measuring to an accuracy of ±0.1 pH until equipped with either manual or automatic temp. Equalization together in a combined electrode system.
Used to measuring the pH of culture media & reagent.

MAINTENANCE AND HANDLING:

It should be calibrated using at least two standard buffer solution, at least daily before use. The standard solution has pH value which are known to be within the second decimal at the measurement temperature (7.0 at 20 °C)
Followed manufactured instruction. The condition of electrodes with respect to again & soiling and the response time and stability monitor regularly.

WATER BATH:

Water will be set and maintained at a specific temperature. Each water bath will be filled with distilled or de-ionized water to which an appropriate amount and concentration of a non-corrosive sanitizer has been added. Each water bath will contain a thermometer calibrated by used of standard/ certified thermometer. The temperature and condition of each water bath shall be checked and documented each day of use. The water level of each water bath must be above the level of the media immersed in the bath.

a. Accuracy should be ±0.5 °C
b. To melt sterile agar media at 47 °C ± 2 °C

Incubate inoculated culture media etc.

MAINTENANCE AND HANDLING:

a. Temperature should be checked each time when it is using.
b. Level of liquid in the bath (water, ethylene glycol etc.)
c. Above proliferation of microbes, liquid should be changed frequency

REFRIGERATOR/FREEZE:

a. Electric circuits used for refrigerator and a freeze are to be rated for the equipment used on that power supply and properly grounded.
b. The laboratory will check all refrigerators (1-8 °C) and freezer (1-20 °C) daily for proper internal temperature. For refrigerators used for sample receipt the temperature range is set at 2-7 °C.
c. The initial temperature of refrigerators will be monitored by use of a appropriate thermometer. Each refrigerator and freeze will be set and maintained at a specific temperature.

The universal biohazard symbol shall be visible to the exterior surface of the freezer or refrigerators when it contains infections microbial culture.

MICROBIOLOGICAL ANALYSIS SHEET

Test parameter	Frequency	Area	Supervised by
Standard plate count	As per requirement	Process line and finished product	QC Inspector
Total coliform	Do	Do	QC Inspector
Fecal coliform	Do	Do	QC Inspector
Salmonella	Do	Do and receive area	QC Inspector
Vibrio cholera	Do	Do and receive area	QC Inspector

MEDIA PREPARATION

Introduction:

Culture media must be stored at the specification temperature, under specified condition and not longer than the shelf-life appropriate to each product. The storage condition and expire date of each product are shown on the labels or product inserts but the following general rules will help to ensure that they are kept in an optimum environment. When storing product note the shelf-life expiry dates on the labels and use the products in order of their lot/batch numbers. The following factors should be considered during media store.

Light:

All prepared culture media and their components should be stored away from light and exposure to direct sunlight should be avoided at all time.

Humidity:

Sealed glass and plastic containers are unaffected by normal laboratory humidity. Opened containers of dehydrated powders will be affected by high humidity. Hot, steamy media preparation rooms are not suitable environments to store containers of culture media, particularly containers which are frequently opened and closed. An adjacent cold room or an adequate storage cupboard is preferable storage areas.

Temperature and Time:

The temperature storage condition of culture media and their components vary widely. The following products grouping will help to differentiate the various requirements.

Culture Media:

Sealed unopened containers should be stored at room temperature 15-20 °C. Opened containers should have the cap or lid carefully and securely replaced. It is important that opened containers are stored in a dry atmosphere at room temperature. Shelf life 1-5 years.

Prepared Both Media:

Store at 2-8 °C. Does not allow the product to freeze. Shelf life 6 month to 2 years.

Prepared plate of culture Media:

Poured plates of agar media are especially vulnerable to infection, dehydration and chemical degradation. Aspheric preparation and storage are essential to protect plate from microbial infection. Water loss on storage can be minimized by impermeable wrapping and/or storage at 2-8 °C. Chemical dehydration e.g. oxidation or antimicrobial loss, can be related by protection from light, heat and dehydration. It is important, however to monitor the storage of prepared plates by quality control tests so that any deterioration can be detected and storage period accurately determine the rate of moisture loss. Weight loss grater than 5% will indicate a significant.

Gas generating kits:

Store at 2-8 °C in a day place. Do not store these kits at a higher temperature for long periods. Shelf life 3 years.

Sterile Reagents:

Store at 20 °C except horse serum store -20 to +8 °C

Susceptibility Discs:

Store at -20 °C but kept working at 2-8 °C. shelf life 1 to 2 years.

Prepared of dehydrated media:

Dehydrated media are hygroscopic and sensitive to moisture, heat and light. They are adversely by drastic change in temperature e.g. hot/cold cycling temperature which may occur between day and night laboratory temperatures in winter.

Storage condition is usually indicated on the products label and should be followed:

1. Write on the label the date of receipt in the laboratory.
2. Store as indicated on the label, usually below 25 °C in a dry area, away from direct sunlight, autoclaves, drying oven or other heat sources.
3. Check expire date on the label, some media have significantly shorter shelf live than others.
4. Use stock in lot/batch number order. Do not open a new bottle until the previous bottle has been emptied. After use make sure the container is tightly closed and return it to the designated area.
5. Order the media in an appropriate size of containers and in a quantity which accords to normal use requirements. A media in a large container which have been opened many times deteriorate on storage. Discard the media if the powder is not free flowing, if the color has changed or if it appears abnormal in any way.

Reconstitution of dehydrated media:

Complete instruction for the preparation of culture media is given on the label of each bottle. As a general rule it tis wise to prepare one-week requirements only.

1. Always use fresh distilled water. Use warm (50 °C) water to hasten the solution of the medium. Rinse all glass-ware with the distilled water and make sure that the vessels are clean and free from toxin chemical which may be absorbed on to the surface of the glass e.g. bile salt, telluride, selenite etc.
2. Prepare the media in a vessel about twice him final volume of the media to allow adequate max 13. Follow the instructions given on the label of each product.

Open the culture media container away from draughts and moister. Avoid inhaling the power and prolonged skin contact. Weigh the powder quickly, accurately and without creating clouds of dust. Recluse the container as soon as possible. Pour half the required volume of distilled water in the vessel, then the weighed quantity of media agitates briskly for a few minutes, pour in rest of the distilled Waterdown the side of the vessel to wash any adherent media back into solution. This is an important step because dry culture media above the level of the water may not be sterilized in the autoclave and may be a source of contamination.

Agar free media will usually dissolve on gentle agitation. Media containing agar should be heated to dissolve the agar before autoclaving. Bring the media to the boil without scorching or burning. Most culture media will require final sterilization in an autoclave at 121 °C for 15 minutes.

Pressure-Temperature relations in Autoclave (figures based on complete replacement of air by system)

Pressure in found	Temperature (°C)	Temperature (°F)
5	109	228
10	115	240
15	121	250
20	126	259
25	130	267
30	135	275

The pH of the dehydrated medium has been adjusted by the manufacture so that the final pH of the prepared media conforms to the label specification. When the media has been cooled to 25 °C do not adjust the pH of dehydrate media prior to sterilization.

Sterilization of culture media:

Sterilization is any process or procedure designs to entirely eliminate viable microorganisms from a material or medium. Sterilization should be confused with disinfection, sterilization, pasteurization or antisepsis which are intended to inactive microorganisms, but may not kill microorganism present. Sterilization can be accomplished by the use of heat, chemical, radiation or filtration.

Sterilization with Heat:

The principal methods of thermal sterilization include i. Moist heat (saturated steam) and ii) dry heat (hot air) sterilization. Heat kills microorganism by protein denaturizing and coagulation. Moist the advantage of being more rapid and requiring lower temperature than dry heat. Moist heat is the most popular method of culture media sterilization. When used correctly, it is the most economical, safe and reliable.

Sterilization Method:

Moist heat Sterilization: water boils at 100 °C, but a higher temperature is required to kill resistant bacterial spores in a reasonable length of time. A temperature of 121 °C for 15 minutes is an accepted standard condition for sterilization up to one liter of culture media. The definition of autoclave at 121 °C for 15 minutes refers to the temperature and time at which the autoclave has been set. The steam pressure of 15 pounds per square inch at this temperature aids in the penetration of the heat into the material being sterilized, if a larger volume is to be sterilized in one container, a longer period should be employed. May factors can affect sterility assurance including size and container of the load and the drying and cooling time, certain products may decompose at higher temperature and longer cycles. For the reason it is important that all loads be properly validated.

The temperature is must reach all parts of the of the load and be maintained for the desire length of time. Recording thermometers are employed for the chamber and thermocouples may be buried inside the load. For the best result when sterilizing culture media, plug tubes

or flasks of liquid with nonabsorbent cotton of cap loosely. Tube should be placed in rakes or packed loosely in baskets. Flashed should never be more than two -third full. It is important to not overload the autoclave chamber and to place content so that there is a free flow of steam around the contents. After sterilizing liquid, the chamber pressure must be reduced slowly to atmospheric pressure. This allows the liquid to cool below the boiling point at atmospheric pressure before opening the door to prevent the solution from boiling.

In adequate operation, all of the air in the air in the chamber must be expelled and replaced by steam; otherwise ``hot spots`` and ``cold spot`` will occur. Pressure temperature relation of a properly operated autoclave is shown in the table below.

Over sterilization or prolonged heating will change the composition of the media. For example, carbohydrate is known to break down in the composition upon overheating. Over sterilizing media can cause a number of problems, including

1. Incorrect pH
2. A decrease in the gelling properties of agar
3. The development of a non-typical precipitate
4. Darkening of the media
5. Loss of nutritive value
6. Loss of selective or different properties

There certain media (e.g. Hektoen Enteric Agar and Violet Red Bile Agar) that should be autoclaved. To dissolved these media formulations, heat to boiling to dissolved completely. It is important to follow all label directions for each media. Media supplements should be sterile and added aseptically to the sterilized media, usually at 45-45 °C.

Dry Heat Sterilization:

Dry heat employed for materials such as metal instrument that could be corrected by moist heat, powders, ointments and dens materials that are not readily penetrated by steam. Because dry heat is effective only at considerably higher temperatures and longer times than moist, dry heat sterilization is restricted to those items, unlike culture media, that will withstand higher temperature. The dry heat time for sterilization is 120 minutes at 160 °C.

Testing Sterilizing Agent:

Sterilization by moist heat (steam), dry heat ethylene oxide and ionizing radiation is validated using biological indicators. The methods of sterilization and their corresponding indicators are listed below:

Sterilization Method	Biological Indicators
Steam	Bacilius stearorhemophilus
Dry heat	Bacilius subtilis var.niger
Ethylene Oxide	Bacilius subtilis var. niger ghobigji
Filtration	Pseudomonas diminuta

For moist heat sterilization, paper stips treated with chemical that change color at the required temperature may be used. The heat-resistant spores of B.stearorhemophilus are dried on paper treated with nutrient media and chemical. After sterilization, the strips are incubated for germination and growth and a color change indicates wherever they have or have not been activated. Spores strips should be used in every sterilization cycle.

Table of faults and possible causes in media sterilization:

Faults: wrong pH value

Possible Causes: pH test carried out above 25 °C. Over heating through prolonged sterilization remitting at 25 °C. Incomplete solution of media. Poor quality of water or containers. Dehydrated media stored incorrectly or beyond the started shelf life.

Faults: Turbidity, perception

Possible Causes: Poor quality water or containers. Overheating or prolonged 50 °C**.** Incorrect pH value. Incomplete the solution.

Faults: Darkening

Passible Causses: Overheating incomplete solution or pH drift. Presence of phosphate in addition of glucose or other sugars and agar.

Faults: Soft gel

Possible Cause: Agar not in solution, poor mixing, prolonged storage at 50 °C. Overheating at low pH values. Error in weighing or over dilution with inoculums or media supplements pH low for agar.

Faults: Poor bacteria growth

Possible Cause: Prolonged and excessive heating, incomplete solution, inhibitory substance in water or containers. Darkening and pH drift.

Preparation of sterilizing media:

Liquid media which are sterilized in their final containers should be cooled down to room temperature as rapidly as possible. Screw caps should then be tightened. Containers of agar media which have been sterilized should be placed in a 50 °C water bath and the media dispensed as soon as it reaches this temperature or within a maximum of 3 hours in the bath. The media should be mixed thoroughly, without bubble formation and aseptically dispensed into sterile containers. Do not expose dishes of agar media to sunlight, it causes excessive condensation on the lid and may cause formation of inhibitory substance by photo-oxidation.

Heat labile supplements should be added to the media after it has cooled to 50 °C. Allow the sterile supplement to come to room temperature before adding it to the agar media. Very cold liquid may cause agar to gel or form transparent flakes which can easily be seen e.g. in blood enriched agar. Mix all supplements into the media gently and thoroughly then distribute into the final containers aw quickly as possible.

Blood used for the prepared of blood agar should be as fresh as possible and should have been store at 2-8 °C (blood not be frozen). Warm blood in a 35 °C incubator before addition to sterile molten agar which has been cooled at (40-45) °C. Adequate mixing in a large head-space vessel is essential to ensure aeration of the blood. Poorly oxygenated blood plates are purplish in color whereas properly aerated bloods agar is cherry-red. Defibrinated blood is recommended for use rather than blood containing an anticoagulant.

Storage of prepared media:

The recommended shelf life of prepared culture media varies consider ability. Screw capped bottle of nutrient broth and agar can be storage for 6 at low ambient temperature (12-16 °C). It is important to store all media away from light. Agar plates should be storage at 2-8 °C in sealed containers to avoid loss of moister. Do not freeze.

Fresh media are better tan storage media therefore avoid long storage times. Some very labile lactam selective agents have very short active lice' s and media containing such substance should be within a few days of preparation.

It is good laboratory practice to establish shelf life for all prepared media and date stamp the containers or holders accordingly. Loss of moisture from agar plates is a common cause of poor bacteriological performance. Do not pre-incubate all plate overnight as a sterility check. Only obviously wet plates require pre inoculation drying.

Ensure that all plates are incubated in a humid environment.

Examine prepared media before inoculation. Look for evidence of contamination, uneven filling or bubbles on surface of agar, color change hemolysis and signs of dehydration such as shrinking, loss of volume. Discard any defective plate or tubes.

Prepare in the use disposal of prepared media:

It should be recognized that inoculation of culture media with bacteria, deliberately or accidently leads to very great numbers being produce. High concentration of any organisms is potentially hazardous and must be disposed of safety by approved method. All infected specimens and inoculated culture media should be handled and finally autoclave before disposal. Any apparatus used and contaminated must be safety disinfected or sterilized; this is particularly important when such apparatus must be serviced or passed out of the laboratory.

The environment in which microbiological cultures are handled must be taken into account. Most containers have categories of organisms which are divided onto those which may be handled tn the general microbiological laboratory, those which are require special laboratory condition and for the most dangerous organisms a totally contained and highly protected environment is required. It may be a criminal offence not to observe rules and regulation. When using culture media always label or identity the container with the specimen details before inoculation. Inoculate the media using aseptic techniques and under the appropriate condition.

Examine the media after incubator for evidence of microbiological growth and carry out the appropriate isolation and identification procedure.

Precautions dehydrated media:

Most of the products supplied have risks expect those usually associated with fine powders. However, to prevent the risk of inhaling fine dust it is recommended that masks should be worn while handling dehydrate media. The mask chosen should perform to the level of British standard no. 6016. The type of mask manufacture by 3M corporation would be suitable for this purpose.

Hazard Dara sheet are available for individual products.

Dehydrated culture media supplied as powders granules or tables should not be eaten. Powder should not be inhaled because irritation of the upper respiratory tract may occur especially with salt products. To avoids mild skin rashes, prevent prolonged contract with the powder. Powders products, If spilled, can be swept up and deposed of in the normal way. Any residue should be washed away with ample cold water.

Hazardous products: Therea are a few products which contain toxin substance and these must be treated with care.

1. **Media containing thallium salts:**
   These products are labeled poison Hallin salt are very toxic by inhalation or by ingestion and there is a danger of cumulative effects products containing thallium salt must be kept away from foods, drink and animals feeding stuff. Always wear a make and gloves when handing the powder.

2. **Media containing sodium aside:**
   These products contain less than 1% sodium aside and have low toxicity. Some persons, However, have enhanced sensitivity to aside therefore could react to accidental exposure to the products. Precautions must be taken to prevent ingestion or inhalation of the dust. Always wear gloves, mask and eye protection. Sodium aside reacts with many metals, specially, copper to produce explosive metal aside. When washing products containing aside down sinks it is essential that sufficient water is used to prevent the powder. Remaining in contact with the pipework and gulleys. The same precaution applies to any biological solution which contains sodium aside as a preservative.

3. **Sodium bi-Selenite:**
   This product is labeled toxic. It is corrosive on contact with skin and produce toxic effects I inhaled or ingested. Teratogenic effects have been suggested.

4. **Cycloheximide:**
   This compound, prepared in supplement vials, reaches a concentration which is considered to be toxic and is labeled accordingly. However, when diluted out into culture media its concentration falls below the minimum level considered to be hazardous. It is important when reconstituting visual containing toxic levels of cycloheximide to ensure that the visual solution does not touch the skin to prevents the creation of aerosols which would allow the compound to be inhaled. Protective gloves and face are advised when using this vials.

5. **User-Laboratory quality control test on prepared media:**
   Quality control test should be carried out by the end-user laboratory to ensure that the performance characteristics of the media are within specification and that the methodology of media preparation is satisfactory.

Each lot/batch of prepared media should be subjected to a minimum testing program which ensures that it is acceptable and will demonstrate a typical bacteria performance.

1. **pH value:** check that pH of the prepared media, when tested in final form at ambient temperature (25 °C) line within the range given on the products labels. The media should be discarded if the pH value lines outside the specified range.

2. **Sterility:** A temperature sample of each lot/batch media should be incubated for at 2-5 days at 30-35 °C and 50-55 °C. as a general rule for a lot of 100 or less units a 3-5% sample should be tested. For a larger lot, 10 microbial growths after incubator. Discard all sterility sample, when the tested have been completed.
3. **Growth performance:** Test the growth support properties of the product by inoculating the media with appropriate stock cultures and fresh isolate. Use a standard inoculation procedure and example the quantitively results obtained. If testing new lots/batch' s of media, inoculate old and new lots in one test and compare the performance of the two lots side by side
4. **Stability:** Periodical perform the above procedures of stored prepared media in order to determine whether the storage condition will given optimal results.
   Agar is a pyrocollodion water soluble polysaccharide extracted from a group of red-purple algae (class Rhodophyceae) including gelidium and gracilaria. These red-purple marine algae are widely distributed throughout the world in temperate zones, Gelidium is the preferred source of agar. The most important properties of agar are. Good transparency in solid and gel forms to allow identification of colony type. Consistent lot gel strength that is sufficient to withstand the rigors of streaking but consistent gelling (32-40 °C) and melting (approximately 85 °C) temperatures a property known as hysteresis essential freedom from metabolically useful chemical such as petri dish, proteins and fermentable hydrocarbons. Freedom from toxic substances (bacterial inhibitors).

V**alue:** Value stand for decimal reduction time and the time required in minute at a specified term produce a 90% reduction in the number of organisms.

Sterilization process: Sterilization process is a treatment process from which the probability of microorganism survival is less than 10^{-6} or one in a million.

Thermal death time and thermal-chemical death time: Thermal death time and thermal - chemical death time are terms referring to the time required to kill a specified microbial population upon exposure to a thermal or thermal-chemical sterilization agent under specified condition. A typical thermal death time value with highly resistant spores is 15 minutes at 121 °C for steam sterilization.

Procedure:

1. Pipetting by mouth must be strictly forbidden.
2. Materials must not be placed in the mouth. Labels must not be licked.
3. All technical procedures should be performed in a way that minimized the formation of aerosols and droplets. The use of hyperemic needles and siring should be limited. They must not be used as substitutes for pipetting devices all spills, accidents and over or potential exposers to infection materials must be reported to the lab in-charge. A written record of such accidents and incidents should be maintained
4. A written procedure for the clean-up of all spills must be developed and followed.
5. Contamination liquids must be decontaminated (chemically or physically) before discharge to the sanitary sewer. An effluent treatment system may be required, depending on the risk assessment for the agents being handled.

Written document that are expected to be removed from the laboratory need to be protected from contamination while in the laboratory.

ANALYSIS FLOW CHART: SPC

Analysis parameter: Standard Plate Count (SPC)

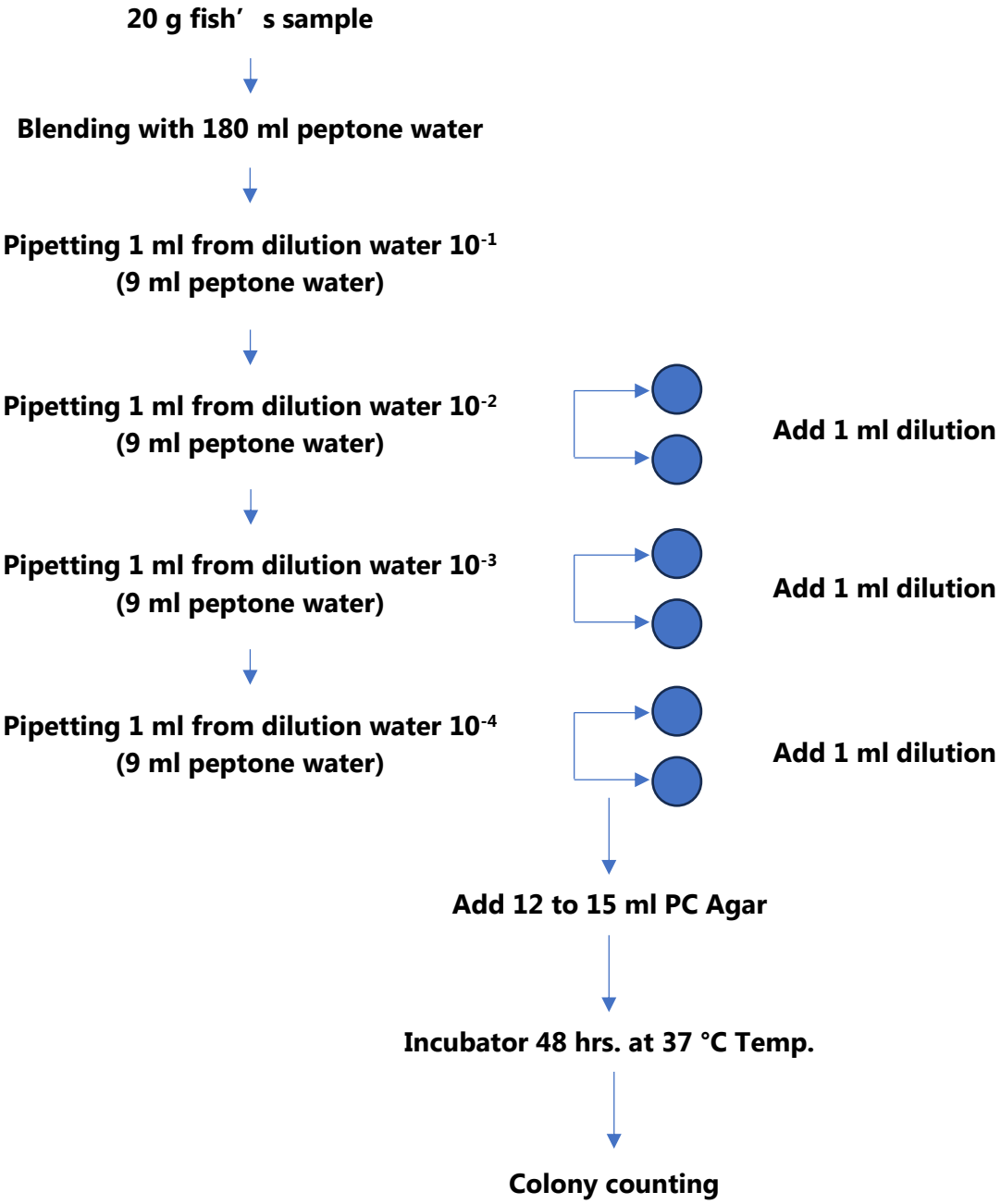


FIG: TEST FLOW CHART OF SPC

Reference: Bacteriological Analytical Manual (BAM)

ANALYSIS PARAMETER OF STANDARD PLATE COUNT (SPC)

Standard Plate Count (SPC):

Description:

1. **Sampling:** A sample unit consist of a minimum of 100 g and usually a consumer size container of products. Take sample unit at random to ensure that the sample is representative of lot. When using sample containers, submit a control consisting of one empty sample container that has been exposed to the sample conditions. Collect the sample from various point at receive to finished products. At the time of sample noted sample number, type of shrimp' s date and time.

2. **Thawing:** Use aseptic technique when handling products. Before handling or analysis of sample, clean immediate and surrounding work areas. In addition, swab immediate work is with commercial germicidal agent. Do not thaw frozen sample before analysis.

3. **Weighing:** Aseptically and accurately the sample must be weighed. If the entire sample weighing less than the required amount, portion equivalent to one half of sample must be weighed and the number of diluents or both must be adjusted accordingly this method. Cut the sample and weighing 20 g digital scale.

4. **Blending & Dilution:** After weighing to blend the sample with 180 ml. peptone water by a electronics blender. Make dilution of original homogenate promptly using pipette that deliver required volume accurately. This is dilution number 10^{-1}. Prepare decimal dilution of 10^{-2}, 10^{-3}, 10^{-4} & 10^{-5} using separate sterile pipettes of 1 ml volume.

5. **Plating and Agar Added:** 1 ml of each dilution is added to one pair sterile petri dish. The dish filled by approximately 12 to 15 ml of PC agar held at 42-45 °C. The containers of the plate are mixed thoroughly and the medium is allowed to solidify. Plates are dried, inverted and incubated.

6. **Incubation:** In incubation all culture plate is incubated at 37 °C for 48 hrs. Plate count agar is used which has been solidified by addition of agar. This is restricting the movement of bacteria which then multiply on incubation to form visible colonies which can be counted.

7. **Colony counting:** After 48 hrs. all plates colony are counting on the colony counter. By using a range of dilution, it is possible to obtain plates with 30 to 300 colonies on them.

OBSERVATION SHEET STANDARD PLATE COUNT (SPC)

Media Name	Duration Time	Incubated Temp.	Dilution Number	Colony Found	Result
PC Agar	48 hrs.	37 °C	**10^{-3}**	<300	
PC Agar	48 hrs.	37 °C	**10^{-4}**	>30	
PC Agar	48 hrs.	37 °C	**10^{-5}**	>30	

Reference: ICMSF -1986

METHOD OF CALCULATION

General case- Dished containing between 30 and 300 colonies.

Retain dishes containing not more than 300 colonies at two consecutive dilutions. It is necessary to disregard the rule above and record the result as observed. With this accepting, only plates shoeing 30 and 300 colonies should be considered in products depending on the case, using the flowing equation.

$$N = \frac{\Sigma C}{(n_1 + 0.1n_2)d}$$

Where,

ΣC is the sum of colonies counted on all the dishes retained.

n_1 is the number of dishes retained in the first dilution.

n_2 is the number of dishes retained in the second dilution.

d is the dilution factor corresponding to the first dilution.

Round the result calculated to two significant figures. Take as the result the number of micro-organisms per millimeter or per gram of products, expressed as a number between 1.0 and 9.9 multiplied by 10^x where x is the appropriate power of 10.

Example:

Micro-organisms count at 37 °C/48 hrs. given the following results.

- At the first dilution retained (10^{-3}): 168 and 215 colonies
- At the second dilution retained (10^{-4}): 31 and 39 colonies

$$N = \frac{\Sigma C}{(n_1 + 0.1n_2)d}$$

$$N = \frac{168+215+31+39}{[2 + (0.1 \times 2)] \times 10^{-3}}$$

$$N = \frac{453}{0.0022}$$

$$N = 2{,}05{,}909$$

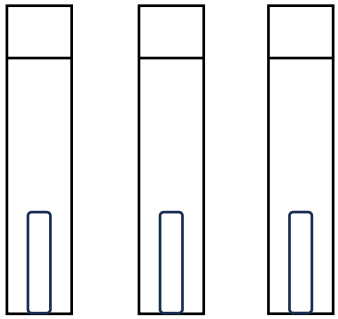
Rounding the result as specification around gives 2,60,000 or 2.6×10^5 micro-organisms per milliliter or per gram of products.

ANALYSIS FLOW CHART OF TOTAL COLIFORM

Analysis Parameter: Total Coliform (After Blending)

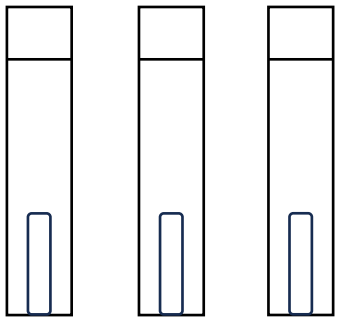
Add 1 ml Dilution per 10 ml LSTB tube

Blending with 180 ml peptone water →



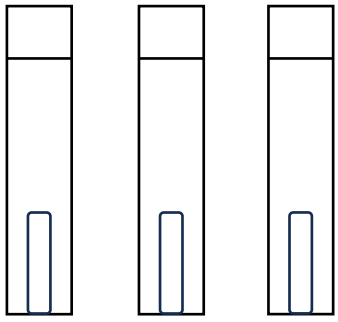
Add 1 ml Dilution per 10 ml LSTB tube

Blending with 180 ml peptone water 10^{-2} (9 ml peptone) →



Add 1 ml Dilution per 10 ml LSTB tube

Blending with 180 ml peptone water 10^{-3} (9 ml peptone) →



All LSTB tubes are incubated at 37°C for 48 hrs. After incubation observed gas formation in all tube and calculate result from MPN chart.

Reference: ICMSF. 2. ISO 7521: 1993

ANALYSIS PARAMETER OF TOTAL COLIFORM

Description:

1. **Blending & dilution:** After weighing to blending the sample with 180 ml peptone water by electronic blender. Make dilutions of original homogenate, using pipette that deliver require volume accurately. This is dilution number **10^{-1}** prepare decimal dilutions of **10^{-2}** & **10^{-3}** using separate sterile pipettes of 1 ml volume.

2. **Pipetting:** Transferred 1 ml dilution of **10^{-1}, 10^{-2}, 10^{-3}** into 10 LSTB test tubes. Then incubate at 37 °C for 48 hrs.

3. **Observation & Result:** After incubation observed gas formation in all tubes and calculate result from MPN chart.

OBSERVATION SHEET OF TOTAL COLIFORM

Media Name	Duration Time	Incubated Temp.	Dilution Number	Gas Found	Result
L. T. B	48 hrs.	37 °C	**10^{-1}**	3	43/gm
L. T. B	48 hrs.	37 °C	**10^{-2}**	1	
L. T. B	48 hrs.	37 °C	**10^{-3}**	0	

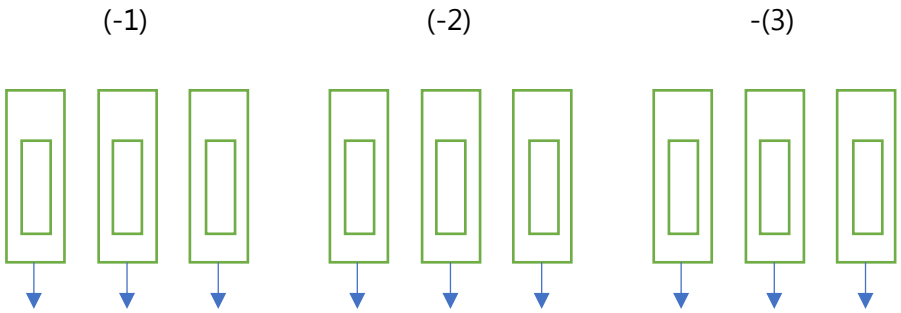
Reference: ICMSF. 2. ISO 7521: 1993

MOST PROBABLE NUMBER TECHNIQUE

Number of Positive Tube			MPN
10^{-1}	10^{-2}	10^{-3}	Per gm
0	0	0	<3
0	0	1	3
0	1	0	3
0	1	1	6
0	2	0	6
1	0	0	4
1	0	1	7
1	1	0	7
1	0	2	11
1	2	0	11
2	1	0	15
2	0	0	9
2	0	1	14
2	1	1	20
2	2	0	21
2	1	2	27
2	2	1	28
2	2	2	35
2	3	0	29
2	3	1	36
3	0	0	23
3	0	1	39
3	1	0	43
3	0	2	64
3	1	1	75
3	2	0	93
3	1	2	120
3	2	1	150
3	1	3	160
3	2	0	210
3	3	1	240
3	3	2	460
3	3	3	1100
3	3		>2400

Source: Bacteriological Analytical Manual Online, January 2001.

ANALYSIS FLOW CHART OF FECAL COLIFORM



All BGLBB & Trypton water tubes are incubated at 45.5 °C for 18 to 48 hrs in circulating water bath. After incubation observed gas formation in all BGLBB tubes. Add 0.2 to 0.3 Kovacs Reagent in Tryptone water tubes and then calculate result from MPN Chart.

ANALYSIS PARAMETER OF FEACAL COLIFORM

DESCRIPTION:

1. **Inoculation:** Identify the gas formation of LSTB test tubes and add 1 drop of LSTB in BGLBB & Tryptone Water tubes. Then incubate in to the circulation water bath 44.5 °C for 18 to 48 hrs.
2. **Observation & Result:** After incubation observed gas formation in all BGLBB tubes, added 0.2 to 0.3 ml Kovacs Reagent in Tryptone water tubes and calculate result from MPN Chart.

OBSERVATION SHEET OF FEACAL COLIFORM

Name of Media	Duration Time	Circulating Water Bath Temp	Dilution No.	Characteristic Ring	Result
BGLBB & T.W	24 Hours	44.5 °C	10^{-1}	2 Deep Red Ring	
BGLBB & T.W	24 Hours	44.5 °C	10^{-2}	0 No Deep Red Ring	
BGLBB & T.W	24 Hours	44.5 °C	10^{-3}	0 No Deep Red Ring	

Number of Possitive Tubes			MPN	Confidence Limit	
10^{-1}	10^{-2}	10^{-3}	Per gm	Lower	Upper

Source: Marvin, L, Spink, 1976

ANALYSIS FLOW CHART OF VIBRIO CHOLERA

Description

1. **Sample:** A sample unit consists of minimum of 100 gm and is usually a consumer size container of products. Take sample unit at random to ensure that the sample is representative of lot. When using sample containers, submit a control consisting of one empty sample container that has been exposed to the same condition, collect the sample from various point at receive to finished products. At the time of sampling, number type of shrimps, date and time.
2. **Thawing:** Use aseptic technique when handling products. Before handling or analysis of sample, clean immediate and surrounding work areas. In addition, Swab immediate work area with commercial germicidal agent. Do not thaw frozen before analysis.
3. **Weighing:** Aseptically and accuracy the sample must be weighed. If the entire sample weighing less that the require amount portion, equivalent to one half sample must be weighed sample and weighing 25 gm by digital scale.
4. **Blending & Diluting:** After weighing to blending the sample with 225 ml, Alkaline Peptone water by an Electronics blender and stored in conical flax.
5. **Incubation:** In incubator all cultures are incubated at 35 °C to 37 °C for 24 hours.

Reference: Bacteriological Analytical Manual (BAM)

OBSERVATION CHART OF VIBRIO CHOLERA

Name of Media	Duration Time	Incubation Temp	Suspected Color	Result
APW Enrichment	18-24 Hours	35 °C to 37 °C	No color change	Not Detectable
TCBS Agar	18-24 Hours	35 °C to 37 °C	Large 2-4 mm. colony	Possibility
TCBS Agar	18-24 Hours	35 °C to 37 °C	Yellow color colony	Possibility
TCBS Agar	18-24 Hours	35 °C to 37 °C	Smooth flattened colony	Possibility
CLEAD	18-24 Hours	35 °C to 37 °C	Color of the colony is transparent	Possibility
TSIA	18-24 Hours	35 °C to 37 °C	Acid slant & Butt (Yellow) and no gas formation	Absent
KIA	18-24 Hours	35 °C to 37 °C	Color of the Butt becomes yellow & Slant turns red	Absent

Reference: Bacteriological Analytical Manual (BAM)

DETECTION PROCEDURE OF VIBRIO CHOLERA

1. **Pre-enrichment:**
   a. Take 25 gm of sample in 225 ml sterile (pre-enrichment media) Alkaline Peptone Water (APW) (1:10) in electronics bag.
   b. Blend for 2 minutes in electronics blender and incubate at 35 °C to 37 °C for 6+1 hours (for raw fish) or 18-24 hours (for cooked fish).

2. **Selective Enrichment Agar Plate:**
   a. Streak a loop full by mean of sterile loop on TCBS agar plate and incubator at 35 °C or 37 °C for 18-24 hours (raw food incubate for 6+1 hours and for cooked foods 18-24 hours (Interpretation: color of the colony. Positive Vibrio cholera colony is yellow smooth oval shape, slightly flat, 2-4 mm).

   b. (Optional) Streak a loop full by means of sterile loop on MCPC (Modified Cellobiose Colistin Polymyxin-B Colistin) agar plate and/ or CC (Cellobiose Coli Tain) agar plate and incubate at 39 °C to 40 °C for 18-24 hours. (Interpretation: Color of the colony: positive colony is small, green to greenish violet/purple, smooth, 1-2 mm, color of the media will be purple /violet in case of long incubation.

Biochemical:

a. TSI (Triple Sugar Iron Agar) Slant: Steak on the surface of slant- slope and stab the butt and incubate at 35 °C or 37 °C for 18-24 hours (Interpretation: Color of the media (Butt and Slant) changed to yellow when positive and no gas formation.

b. Klinger Iron Agar Slant: Steak on the surface of slant-slope and stab the butt becomes yellow and no gas formation and slant turns red when positive.

c. (Optional): Arginyl glucose slant (L-arginyl De hydrolase test): Streak on the surface of slant-slope and stab the butt and incubate at 35 °C or 37 °C for 18-24 hours (Interpretation: color of the butt becomes yellow (acidic reaction)) and no gas no hydrogen sulfite formation and slant turn purple/violet (alkaline reaction) indicates position.

d. CLEAD Agar Plate: Streak on the plate from TSI agar incubates at 35 °C to 37 °C for 18-24 hours (Interpretation: color of the colony is transparent indicates positive).

3. **Salt Tolerance Test:**

Inoculate Tryptone Broth (T1N0) and Tryptone salt (T1N1 1% NaCl, T1N2 2% NaCl, T1N3 3% NaCl, T1N8 8% NaCl) by with a sterile loop and incubate at 35 °C or 37 °C for 18-24 hours. Better Growth of Vibrio will be observed in the lower concentration of NaCl, not in 8% NaCl.

Indole Test (Kovac Reagent):

With sterile loop, inoculate Tryptone Water Broth (1%) from TSI Agar/nutrient/TCBS Agar and incubate at 35 °C or 37 °C for 18-24 hours. Add 0.2-0.3 ml Kovac Reagents (Optional: gentle shaking). (Interpretation: a dark pink/red color at the same surface indicates positive test (brownish color indicates negative test). Kovacs Reagents: 1.0 gm p-dimethyl amylobzal-dehyed is dissolved in 15 amyl alcohol and then slowly add 5.0 ml conc. Hydrochloric acid.

String Test: 0.5% sodium deoxycholate solution

Suspend a Vibrio cholera colony by sterile platinum loop in once drop 0.5% sodium deoxycholate solution on a slide, with in 10 sec the colony become transparent (loose turbidity, cell breaks down) and DNA comes out of the cell and sodium becomes strike and steering like structure.

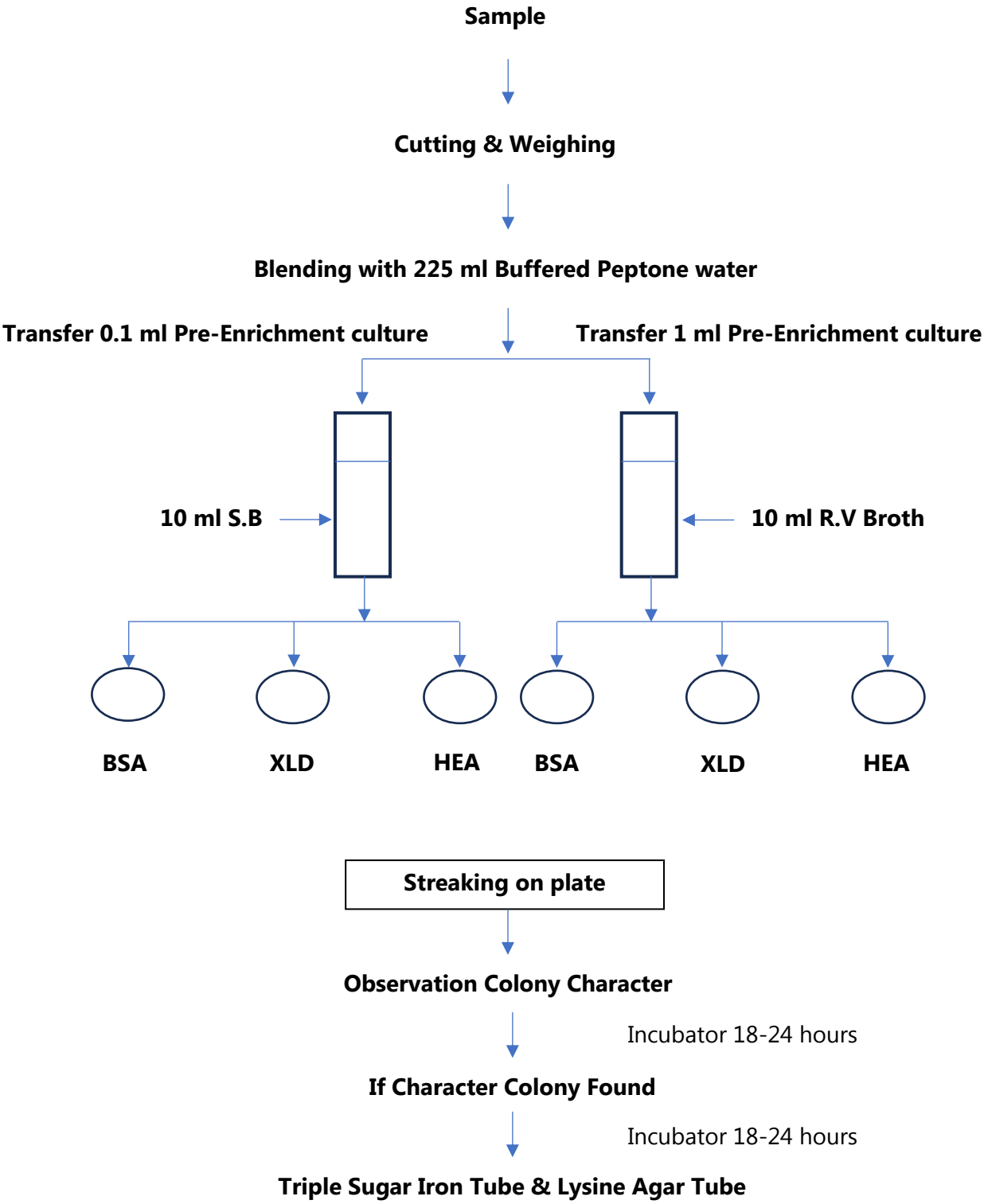
Oxidase test (1% NNN`N- Tetramethyl-p-phenyl diamine hydrochloride)

Soak Whatman filter paper with few drops of 1% NNN`N -Tetramethyl-p-phenyl diamine hydrochloride solution and steak by means of the sterile platinum loop or glass rod a vibrio cholera colony from Nutrient Agar Plate/Slant, the color of the filter paper changes first to pink then purple which indicates the positive test (usually, enter bacteria cease family gives negative test (on changed in color).

Serum agglutination:

Take on drop of sterile physiology saline (0.85% NaCl solution) onto a clean glass slide and pink a single colony and make a homogenous suspension and title the slid and for $^{1}/_{2}$ min and observe against a dark background for any kind of agglutination. Glandular particles (agglutination) indicate positive reaction.

ANALYSIS FLOW CHART OF SALMONELLA



Reference: **Bacteriological Analytical Manual (BAM)**

ANALYSIS PARAMETER OF SALMONELLA

Description:

1. **Sampling:** A Sample unit consists of a minimum of 100 gm and usually a consumer size container of products. Take sample unit of ensure that the sample unit to ensure that the sample is representative of a lot/consignment. When using sample containers, submit a control consisting of on empty sample from various point at receive to finished products. At time of the sampling note sample number, type of sample, date and time.
2. **Thawing:** Use aseptic technique when handling products. Before handling or analysis of sample, clean immediate and surrounding work areas. In addition, Swab immediate areas with commercial germicidal agent. Do not thaw frozen sample before analysis.
3. **Weighing:** Aseptically and accuracy the sample must be weighing. If the entire sample weighing less than the required amount, portion equivalent to one-half of sample must be weighed and the amount of diluent`s or broth must be adjusted accordingly. Accordingly, this method cut the sample and weighing 25 gm by digital scale.
4. **Blending & Diluting:** After weighing to be blend the sample with 225 ml Buffered Peptone water (Pre-enrichment) by an electronics blender and stored on conical flax.
5. **Incubation:** Every step of test cultures is incubated in incubator at 35 °C to 37 °C for 18-24 hours.
6. **Streaking:** 1 loop of enrichment broth SB & RV culture streaking on XLD, BSA & HEA plates. At TSI tube streaking first on slant and stubbing last in butt. At LIA stubbing double at first in butt and streaking last on slant.
7. **Observation:** After incubation procedure colonies size color, stricture, character observed every step of test.

OBSERVATION SHEET OF SALMONELLA

Name of Media	Duration	Incubation Temp	Suspected Color	Standard/ Limit	Result
BPW Pre-Enrichment	18-24 Hours	35 °C to 37 °C	No	Absent	Not detectable
Selenite Enrichment	18-24 Hours	35 °C to 37 °C	No	Absent	Not detectable
RV Enrichment	18-24 Hours	35 °C to 37 °C	No	Absent	Not detectable
S-HEA R-HEA	18-24 Hours	35 °C to 37 °C	Bluish-green colony	Absent	Possibility
S-BSA H-BSA	18-24 Hours	35 °C to 37 °C	Black with Metallic-shine colony	Absent	Possibility
S-XLD H-XLD	18-24 Hours	35 °C to 37 °C	Pink or Red colonies with or without black center	Absent	Possibility
TSAI Slant	18-24 Hours	35 °C to 37 °C	Alkaline slant (Red) lactose & sucrose not fermented	Absent	Absent
TSIA Butt	18-24 Hours	35 °C to 37 °C	Alkaline Butt (Red) Dextrose not fermented	Absent	Absent
LIA Slant	18-24 Hours	35 °C to 37 °C	Alkaline Purple/Red Butts	Absent	Absent
LIA Butt	18-24 Hours	35 °C to 37 °C		Absent	Absent

Reference: Bacteriological Analytical Manual (BAM)

DETECTION PROCEDURE OF *SALMONELLA* spp

1. Pre-enrichment
   - Take 25 gm of sample in 225 ml sterile (pre-enrichment media) buffered peptone water (BPW) (1:1) in electronics bag.
   - Blend for 2 min electronics Blender and incubate at 35 °C to 37 °C for 18-24 hours
2. Prepare culture
   - Add 0.1 ml of pre-enrichment culture to 100 ml Rappaport Vassiliadis Magnesium chloride- malachite green broth (Sterile RV medium) and incubate at 42±2 °C for 18-24 hours. (If needed further 24 hours).
   - Add 0.1 ml (10 ml) of pre-enrichment culture to 10 ml (or 100 ml) selenite cystine broth. (Sterile) and incubate at 35 °C or 37 °C (if 42 °C± 2 °C mention in report) for 18-24 hours. (If needed further 24 hours).
3. Steak one loop full on enrichment Agar plate:
   - Phenol red/Bag (Brilliant Green Broth): Incubate at 35 °C or 37 °C for 18-24 hours (Interpretation: The color of the media change from pink to red)
   - XLD Agar: Incubate at 35 °C or 37 °C for 18-24 hours (Interpretation: Colony -pink {Red} with or without black center)
   - HEA Agar (Hektoen Enteric Agar): Incubate at 35 °C or 37 °C for 18-24 hours (Interpretation: Colony -Blue Green to blue with or without black center)
   - BSA (Bismuth Sulphite Agar): Incubate at 35 °C to 37 °C for 48 hours (Interpretation: Characteristic colony (5 colonies) -Black with metallic shin) Optional: Streak suspected colony (5 colonies) on nutrient Agar.

Biochemical Tests:

Agar Slants: TSI (Triple Sugar Agar) Slant: Stab the Butt and Streak on the agar slope surface a suspected colony with sterile platinum needle and the loosely capped slant is incubated at 35 °C to 37 °C for 18-24 hours (Interpretation: color of slant surface-

- Alkaline Red or unchanged lactose and sucrose negative, glucose negative -no fermentation of glucose.
- Yellow slant surface and sucrose positive. Color of Butt Acid Yellow Glucose Positive - Fermentation of glucose or black butt indicate H_2S gas formation.
- Bubble or cracks indicates gas formation for glucose.

Result: Typical Salmonella culture shows alkaline (Red) slant with gas formation and acid (yellow) butt, with (in about 90% cases) formation of H_2S (Blackening of the agar)

When a lactose -positive salmonella is isolated, the TSI slant is yellow, Thus the preliminary confirmation of salmonella culture shall not be based on the result of TSI agar test only.

1. **LIA (Lysine Iron Agar) Slant:** Stab the Butt and streak on the agar slop surface a suspected colony with sterile platinum needle and the tightly capped slant incubated at 35 °C to 37 °C for 24 hours.
2. **Color of Butt:** Alkaline purple/red butts indicate a positive test with H_2S gas production and yellow butts indicate a negative test.
3. **Urea Agar Slant:** Streak only on the slope surface from TSI or Nutrient agar and incubated at 35 °C to 37 °C for 18-24 hours. (Interpretation: Color of the slant- positive

reaction indicates the color of media changed from phenol red to rose pink and late to deep cerise (Light clean red) as urea is converted to ammonia and some time the reaction takes place with 2-4 hours.

4. **MacConkey Agar Plate:** Streak only the agar surface from TSI or nutrient agar and incubate 35 °C to 37 °C for 6-24 hours. (Interpretation: color of colony- The transparent colony with opaque center indicates the positive test.

Serum Aqqlutination:

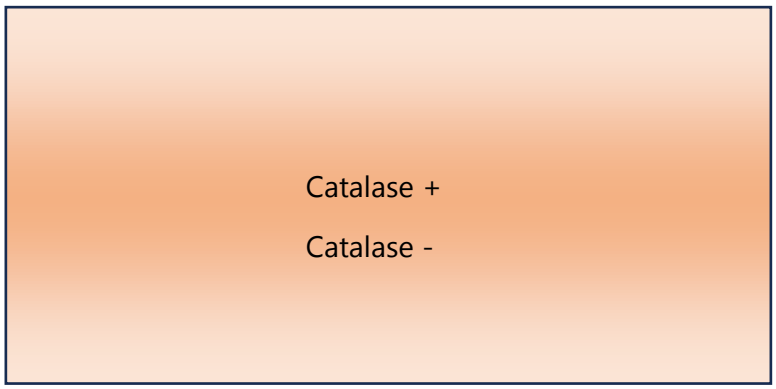
Take one drop of sterile physiological (0.85% NaCl solution) into a clean glass slide and pick a single colony from the agar plate with a sterile glass rod a platinum loop (do not use nichrome loop or iron loop which may give false positive result) and make a turbid and homogenous suspension, till the slide for 2-3 min and observe for kind of agglutination (Small particular object or granular particles. This services as blank of negative control).

Similarly, place a drop (10-12) of specific antiserum at room temperature on a slide and pick the same colony and make a homogeneous suspension and till the slide for 1-2 min and observe against a dark black ground for any kind of agglutination. Granular particles (agglutination) indicate positive reaction.

BIOCHEMICAL TEST:

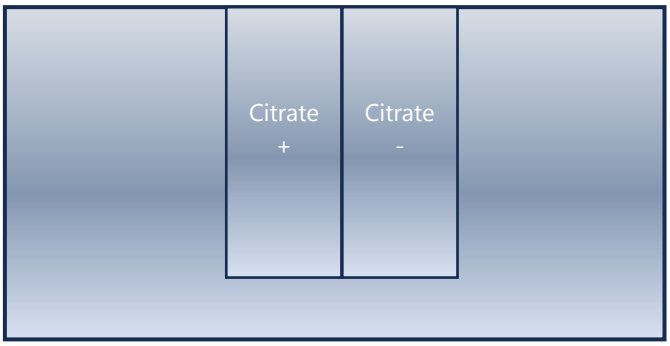
CATALASE TEST:

Some bacteria and macrophage can reduce diatomic oxygen to hydrogen peroxide or super oxide. Both of these molecules are toxic to bacteria. Some bacteria, However, possess a defense mechanism which can minimize the harm done by two compounds. These resistant bacteria use two enzymes to catalyze the conversion of hydrogen peroxide and super oxide black into diatomic oxygen and water. One of these enzymes is catalase test involves adding hydrogen peroxide to a culture sample test slant. If the bacteria in question produce catalase, they will convert the hydrogen peroxide and oxygen gas will be involved. The evolution of gas cause bubbles formation and indicate as a positive test.



CITRATE TEST:

The citrate is used to determine the ability of a bacterium to utilize citrate as its only source of carbon. Bacteria can break the conjugate base salt of citrate into organic acids and carbon dioxide. The carbon dioxide can combine with the sodium from the conjugate base salt to from a basic compound, sodium carbonate. A pH indicator in the medium detected the presence of this compound by turning blue. (A positive test)



OXIDASE TEST:

Cytochrome oxidase is an enzyme found in some bacteria that transfers electrons to oxygen, the final electron acceptor in some electron transport chains. Thus, enzyme oxidizes reduced cytochrome c to make this transfer of energy. Presence of cytochrome oxidase can be detected through the use of an Oxidase disk which acts as an electron donate to cytochrome oxidase. If the bacteria oxidase the disk (remove electron) the disk will turn purple, indicating a positive test. No color change indicates a negative test.

INDOLE TEST:

Indole is a component of the amino acid tryptophan. Some bacteria have the ability to back down tryptone for nutritional needs using the enzyme tryptophan. When tryptophan is broken down, the presence of indole can be detected through the use of Kovac' s reagent, which is yellow, reacts with indole and procedures a red color on the surface of the test tube.

UREASE TEST:

Urease is an enzyme that breaks the carbon-nitrogen bond of amides to form carbon dioxide, ammonia and water. Members of genus Proteus are known to produce urease. Urease can be detected by plating bacteria on to an amide containing medium, specifically urea. When urea is broken down, ammonia is released and pH of the medium increases (become more basic). The pH change is detected by pH indicator that turns pink in a basic environment. A pink medium indicates a positive test of urease.

TRIPLE SUGAR IRON (TSI) AGAR:

Triple sugar iron agar is a medium used in the identification of gram-negative enteric rods. The medium measures a bacterium`s ability to utilize three sugar glucose, sucrose, lactose, the concentrations of which are 0.1% and 1.0% respectively. A pH indicator included in the medium can detect acid production from fermentation of these carbohydrates. A yellow color change indicators acid in the medium while no color change indicates an alkaline surrounding. Inoculation of the tube is a two-step procedure. First a loop of bacteria is spread across the surface of the agar.

Second, a needle of bacteria is inserted (stabbed) into the bottom (butt) of the tube. Carbohydrate utilization can be determining trough analysis of the extent of acid production. Acid production limited to only the butt of the tube is indicative of glucose utilization. This is because the concentration of glucose is lower than other sugar, thus the acid production is not very extensive. Acid production in the slant and butt indicates sucrose or lactose fermentation because of very relatively high concentration of these sugars, thus leading to thiosulphate to hydrogen sulfide. Hydrogen sulfide production will turn parts of the agar black. Production of others gases id marked by cracks in the agar as well as an air gap at the bottom of the test tube.

INTERPRETATION					
	Tube-01	Tube-02	Tube-03	Tube-04	Tube-05
Slant	-	A	A	K	K
Butt	-	A	A	A	A
Hydrogen Sulphite	-	-	-	+	+
Gas	-	-	-	-	-
A=Acidic K=Alkaline					

LABORATORY MANUAL

WATER & ICE TEST

WATER & ICE

Introduction:

For monitoring the sanitary condition and hygienic practices of Fish Processing plants, test sample should be drawn at prescribed intervals.

It includes:

- Water used for fish processing
- Ice used for fish processing

Bacteriological offers the most delicate test for the detection of recent and therefore potentially dangerous fecal pollution; this is the chief function of the bacteriological examination.

The impression of security given by bacteriological testing of water at lengthy intervals may, therefore, be quite false. Indeed, the value of bacteriological tests in depends upon their frequent and regular application.

It is far more important to examine a supply frequently by a simple test than occasionally by a more complicated test or series of test.

BACTERIOLOGICAL EXAMINE OF WATER & ICE

Media Preparation Utensil:

Use borosilicate glass or other suitable non-corrosive equipment such as stainless steel. Glassware must be clean and free from foreign residues or dried particles of agar and also from toxic or foreign materials that may contaminate media, such as chlorine, copper zinc, antimony, chromium or detergents.

Pipettes and Graduate Cylinders:

Pipettes may be of any convenient size provided they deliver accurately and quickly the required amounts.

Pipette Containers:

Boxes shall be of aluminum or stainless steel. Copper or copper alloy cans or boxes should not be used as pipet containers.

Dilution Bottles:

Bottles or Tubes shall be resistant glass, preferably borosilicate glass closed with stoppers or screw caps equipped with liners.

Petri dish:

For making the standard plate count, use petri dishes about 100 mm in diameter with the side wall of bottom at least 15 mm high. The bottom of the dish shall be free from bubble and scratches and shall be flat so that the medium will be of uniform thickness throughout the plate.

Fermentation Tube and Vials:

Fermentation tubes of any may be used. Where tubes are to be used for a test of gas production, enclosed a shall vial inverted. The size of the tube and the vial shall be such that the vial is completely filled with medium and at least partly submersed in the tubes.

Inoculation and Equipment:

Wire loops shall be made of 22- or 24-gauge nichrome or platinum-iridium where flame sterilization is used. The diameter of all loops shall be at least 3 mm.

Sample Bottle:

Bottles of glass or other materials resistant to the solvent action of water capable of being sterilized and of any suitable size and sharp may be used for samples intended for microbiological examination, bottle shall hold a sufficient volume of samples all the required tests, permit proper washing, and maintain the samples uncontaminated until the examination is completed.

Ground glass stopper bottles preferable wide-mouthed and of resistant glass are recommended. Before sterilization, cover the top and necks of sample having glass closures with metal foil, rubberized cloth. Heavy impermeable paper. Many plastic sample bottles are, commercially available. These should not be used for repetitive sampling unless it has been demonstrating that they can be sterilized.

WASHING AND STERILIZATION

Introduction:

Clean all glassware thoroughly with suitable detergent and hot water, rinse hot water to remove with all traces of residual washing compound and finally rinse distill water.

Sterilization:

Glassware expects when in containers, shall be sterilization for not less than 60 min at a temperature or 170 °C for not less than 2 hours.

Preparation of Culture Media:

Storage of culture media: Store dehydrated media (powders) in tightly closed bottles in the dark at less than 30 °C in an atmosphere of low humidity. Do not use then if they discolor or become caked so as to lose their free-flowing power. It is advisable to purchase dehydrated media in small quantities that will be used within 60 months after opening.

Additionally, Stocks of dehydrated selective media should be of relatively current lot number (within a year of purchase) so as to maintain optimum selectivity.

Adjustment of Reaction: The reaction of culture media should be stated in timers of hydrogen ion concentration, expressed as pH. The increase in hydrogen ion concentration (decent ratio in pH) during sterilization will heavy slightly with the individual sterilization in use, and the initial reaction.

Make the tests to control the adjustment to the required hydrogen ion concentration with a pH meter. Calculation the amount if NaoH solution that must be added to the bulk of the medium to reach this reaction. After addition and thorough mixing check the reaction and adjust if necessary. The required final pH is given in the directions for preparing each medium.

Sterilization: Sterilize all media except sugar broths or broths with other specification in an autoclave at 121 °C. When the pressure reaches zero remove the medium from the autoclave.

Media specification: Never prepare media from the basic ingredients when suitable dehydrated media are available.

Lauryl Sulfate Tryptose Broth (LSB), Brilliant Green Lactose Bile Broth (BGLBB), Tryptone Broth etc. is commercially available in the dehydrated form. Follow the manufacture' s direction for dehydrations and sterilization.

Dilution Water: Peptone dilution water, prepare a 10% solution of peptone in distilled water. Dilute a measured a volume to provide a final 0.1% solution. Final pH should be 6.8.

SAMPLES:

Collecting Container: Sample for bacteriological examination must be collected in bottles that have been cleaned and rinsed with great care, given a final rinse with distilled water and sterilized properly.

De-chlorination: A De-chlorination agent should be added to bottles intended for the collection of water containing residual chlorine. Sodium thiosulfate is a satisfactory dichlorination agent. Its presence at the instant of collection of the sample from a

dichlorination of bactericidal of the chlorine during the time the sample is in transit to the laboratory. The bacteriological examination will then indicate more probably the true bacterial content of the water at the time sampling.

The sodium thiosulfate should be added to the clean sample bottle before sterilization in an amount sufficient to provide an approximate concentration of 100 g/ 1 sample. This can be accomplished by adding to a 120 ml bottle 0.1 ml of a 10% solution of sodium thiosulfate (this will neutralize) a sample containing about 15 mg of residue chlorine. The bottle is then stoppered capped and sterilization by either dry or moist heat.

Sample Procedure: When the sample leave air space in the bottle (at least 2.5 cm or 1 in) to facilitate. Mixing of the sample by shaking, Preparatory to examination. Care must be exercised to make samples will be representative of water being tested and to avoid contamination of the sample at the time of collection or in the period before examination. The sampling bottle shall be kept un-opened unit the moment it is to be filled. Remove the stopped and hood or cap as a unit taking care to avoid soiling. During sampling do not handle the stopped or cap and neck of the bottle and protect them from contamination. Hold the bottles near the base fill it without rinsing, replace the stopped or cap immediately and secure the hood around the neck of the bottle. If the sample of water is to be taken from a distribution system tap, the tap should be opened fully and the water allowed running to waste for 2-3 min. After turning off the tap, the outer surface should be dried or a lighting a piece of control wool soaked in mentholated sprit and holding it with tongs close to the nozzle, unit the whole tap is hot. The tap should be cooked by allowing water to run to waste for a few seconds and the sample bottle filled from a gentle stream of water, care being taken to avoid splashing.

Size of the Sample: The volume of a sample should be sufficient to carry out all the tests required. Preferable not less than 250 ml of water for samples intended for bacteriological examination.

Identifying Data: All sample should be accompanied by complete and accurate identifying and descriptive data.

Preservation and Storage: The bacteriological examination of a water sample should be started promptly after collection to avoid predictable changed. If sample can not be processing within 1 hour after collection, the use of iced coolers for storage of water samples during transport to the laboratory is recommended.

STANDARD PLATER COUNT

Introduction:

The standard plater count procedure provides a standard sized means of determining the density of bacteria in water. This is an empirical measurement because bacteria occur singly, in pairs chains, cluster or packets and no single growth medium or set of physical and chemical conditions can satisfy the physiological requirement of all bacteria in a water sample. Consequently, the number of colonies may be lower substantially than the actual number of viable bacteria present. To facilitate the collection of reliable data for water quality control measurement especially for comparative and legal purpose a standardized plate count procedure is essential.

Work Area:

A level table or bench top with sample area available in a clean, dirt free, well-lighted room. Table and bench top should be a nonporous surface and should be disinfected before any analysis is conducted.

Sample:

Potable water sample from a distribution system should be collected. Initial sample analysis as soon as possible to minimize changes in the bacterial population. The recommended maximum elapsed time between collection and examination of unrefrigerated sample in 8 hrs.

(Maximum trains time 6 hours, maximum processing time 2 hours). When analysis cannot be within 8 hours maintains the sample at a temperature below 10 °C. The maximum elapsed time between collection and analysis shall not exceed 30 hours.

Sample preparation:

Make each plate with sample number, dilution, date and any others necessary information. Prepared duplicate plates for each volume of sample or sample dilution examination.

Sample Dilution:

a. Selecting dilutions: Select the dilution(s) so that the total numbers of colonies on a plate will be between 15 to 300. For example, a standard plate count as high as 3000 may suspected, prepare containing 1:100.

For most potable water samples, plate count suitable for counting will be obtained by planning 1 ml and 0.1 ml undiluted sample.

Reference: ISO 6222: 1999(E)

WATER & ICE SAMPLE TEST PROCEDURE

Transfer 0.1 ml water sample to each petri dish- add 15 to 20 ml molten Plate Count Agar (44 °C to 46 °C) in each petri dish.

- Mix well and allow temp set on a level surface.
- Incubate the plates at 37 °C for 48 hours or 22 °C for 72 hours.
- Remove the plate from the incubator at the end of 48 hours/ 72 hours depending on the incubator temperature.
- Count the number of colonies of the plates and record it
- Calculate the number of colonies per ml of water sample.
- Report as the numbers of colony forming units per ml of water. Also mention the incubation time and temperature in the report.

a. Measuring sample portions: Use a sterile pipette for initials and subsequent transfers from each container. If the pipette becomes contaminated before the transfer are completed, replace it with a sterile pipette. Use a separate sterile pipette for transfers from each different dilution.

Do not prepared dilutions and pour plates in directs sun light.

- Use caution when remove sterile pipettes from the containers to avoid contamination of the pipette.
- When removing sample, do not insert pipette more than 2.5 cm (1 inch) below the surface of the sample or dilution.

b. Measuring Dilution: When measuring diluted sample of water, hold the pipette at an angle of about 45 °C with the tip touching the side cover of the petri dish or the inside neck of the dilution bottle. Lift the cover of the petri dish.

Enough to insect the pipette. Allow 2 sec for the liquid to drains from the pipette to the petri dish.

Planting:

a. **Melting medium:** Melt sterile agar medium in boiling water or exposure to flowing steam in a partially container, but avoid prolonged exposure to unnecessarily high temperature during and after melting. Do not desterilize the plating medium. Discard melted agar thar contains precipitate.

Temper the melted medium in a water bath between 44 °C to 46 °C unit used. In a separate containers place a thermometer in water or medium that has been exposed to the same heating and cooling as the plating medium. Do not depend on the sense of the touch to indicate the proper temperature of the medium when pouring agar. Use standard plate count agar.

b. **Pouring the plates:** Limit the numbers of sample to be plated in any on series so that no more than 20 min (preferably 10 min) elapse between dilutions of the first sample and

pouring of the last sample in the series. Pure at least 15 and 20 ml of liquefied medium at 44 °C and 46 °C into each plate gently lifting the covers of the medium in the dish just high enough to pour the medium. Carefully plate lid when pouring. As is plate is poured, mix the melted medium thoroughly with the test portion in the petri dish first in one taking care not to splash mixture over the edge by rotating the dish in one directing and then in the opposite directing or by rotating and tilting dish.

Allow the plates to solidify (within 10 min) on a level surface. After the medium solidifies, invert the plates and place them in the incubation.

c. **Sterility Control:** Check the Sterility of the medium and the dilution water blanks by pouring control plates for each series of samples. Additional control also may be prepared to determine contamination of plates, pipettes and room air.

Incubation:

Incubate for the standard plate count for all water samples except bottled water at a temperature 37 °C for 48 hours or 22 °C 72 hours.

Plates should be packed without crowding in the incubator. Any deviation from this must be stated in the examination report.

Counting and Recording:

Promptly count all colonies after incubation period. If counting must be delayed.

Temporarily store the plates at 5 °C to 10 °C for period of on more than 24 hours, but avoid this as routine practice. Record the result of sterility controls on the report for each lot of samples. Ordinarily, it is not desirable to plate more than 1.0 ml of water in a plate therefore.

When the total number of colonies developing from 1.0 ml is less than 15, it is necessary to disregard the rule above and record the result as observed. With the exception, only plates showing 15 to 300 colonies should be considered in determinig the standard plate count.

Method of calculation:

General case-Dishes containing between 15 and 300 colonies.

Retain dishes containing not more than 300 at two consecutive dilutions. It is necessary to disregard the rule above and record the result as observed. With this accepting, only plates showing 15 to 300 colonies should be considered in products depending on the case, using the following equation:

$$N = \frac{\Sigma C}{(n_1 + 0.1n_2)d}$$

Where:

ΣC is the sum of colonies counted on all the dishes retained.

n_1 is the number of dishes retained in the first dilution.

n_2 is the number of dishes retained in the second dilution.

d is the dilution factor corresponding to the first dilution.

Round the result calculated to two significant figures. Take as the result the number of micro-organisms per millimeter or per gram of products, expressed as a number between 1.0 and 9.9 multiplied by 10^x where x is the appropriate power of 10.

Example:

Micro-organisms count at 37 °C/48 hrs. given the following results.

- At the first dilution retained (10^{-2}): 168 and 215 colonies
- At the second dilution retained (10^{-3}): 31 and 39 colonies

$$N = \frac{\Sigma C}{(n_1 + 0.1n_2)d}$$

$$N = \frac{168+215+31+39}{[2 + (0.1 \times 2)] \times 10^{-3}}$$

$$N = \frac{453}{0.022}$$

$$N = 20{,}590$$

Rounding the result as specification around gives 20,500 or 2.05×10^4 micro-organisms per milliliter or per gram of products.

Estimation of small numbers

If two dishes corresponding to the test sample (liquid products) or the initial suspension (other products), contain less than 15 colonies calculator the arithmetic mean m of the colonies counted on both dishes.

Report the result as following:

- ✓ Estimated numbers of micro-organisms per gram.
  NE=m (liquid products)
- ✓ Estimated numbers of micro-organisms per gram
  NE=m×d (other products), where d is the dilution factor of the initial suspension

Report as the Estimated plate count per milliliter.

No colonies:
If the two dishes corresponding to the test sample (liquid products) or the initial suspension (other products) contain no colonies, report the result as follows:

-Less than 1 micro-organism per milliliter (liquid products)

-Less than 1 micro-organism per gram (others products), where d is the dilution factor of initial suspension.

Computing and recording counts:

To compare the standard plate count, multiply the total number of colonies or the average numbers (if duplicate plates of the same dilution) per plates by the reciprocal of the dilution used. Record the dilution used and the number of colonies on each plate counted or estimated.

When the colonies on duplicate plates and or consecutive dilution are counted and the result are average before being recorded, round off counts two significant figures only at the time of conversion to the standard plate count.

Avoid creating fictious ideas of precision and accuracy when computing standard plate count by recording the two left hand digits. Raise the second digit to the next highest number only when the third digit from the left is 5, 6, 7, 8 & 9; use zeroes for each successive digit toward the right from the second digit. For example, a count of 142 is recorded as 140, and a count of 155 as 160, when as a count of 35 is recorded as 35.

Report Count:

Reporting count as "Standard Plate Count "per milliliter.

Personal Error:

Avoid inaccuracies in counting due to carelessness, damaged or dirty optics that impair vision, or failure to recognize colonies. Laboratory workers who can not duplicate their own counts on the same plate within 5% and the count of others analysis with 10% should be discover the cause and correct such disagreements.

MULTIPLE-TUBE FERMENTATION TECHNIC FOR MEMBERS OF THE COLIFORM GROUP

The Standard Plate Count test for coliform group may be carried out by the multiple-tube fermentation technique.

Water of Drinks Water Quality (Potable water):

For the routine examination of most potable water supplies, particularly those that are disinfected, the object of the test is to determine the presence or absence of coliform organism as a measure of either efficiency of operation or the presence of bacterial contamination.

Standard Total Coliform MPN Tests:

Presumptive Test:

Lauryl Tryptone Broth (LTB)/Lactose Broth/ MacConkey`s Broth may be used in the presumptive Test.

Procedure:

Inoculation a series of fermentation tubes (Primary fermentation tubes) with appropriate quantities of the water to be tested.

50 ml water sample in 50 ml (DS) LTB Tube.....in 1 tube

10 ml water sample in 10 ml (DS) LTB Tube.....in 5 tubes

01 ml water sample in 10 ml (SS) LTB Tube.....in 5 tubes

Mix thoroughly after adding the water sample to the LTB broth in the fermentation tubes.

Incubation:

Incubate the inoculate fermentation tubes at 37 °C for (24 hrs. + 24 hrs. + 24 hrs.) total 72 hrs.

Observation:

Observed for gas production in the inverted Durham' s tube in the fermentation tubes. At the end of 24. Shake each tube gently and examine it and if no gas formed and been trapped in the inverted vial, repeat this step at the end of 48 hrs. and 72 hrs. Record the presence or absence of gas formation at each examination of the tubes, regardless the amount.

Interpretation:

Formation of gas within 72 hrs. of gas in any amount in the inner fermentation tubes or vials constitutes a positive presumptive test.

The appearance of an air bubble must not be confused with actual gas production. If the gas formed as a result of fermentation, the both medium will become cloudy. Active fermentation may be shown by the continued appearance of small bubbles of gas throughout the medium outside the inner vial when the fermentation tube gently shaken.

The absence of gas formation at the end of 72 hrs. incubation constitutes a negative test.

Confirmed Test:

Use Brilliant Green Lactose Bile (2%) broth (BGLBB) fermentation tubes for the confirmed test.

Procedure with Brilliant Green Lactose Bile (2%) Broth:

Gently Shake or rotate primary fermentation tubes shoeing gas with a sterile metal loop, transfer one loop of medium (culture) to a fermentation tube containing Brilliant Green Lactose Bile (2%) broth.

Incubation:

Incubate the inoculated brilliant green lactose bile (2%) broth tube for 48 hrs. at 37 °C.

Observations:

The fermentation of gas in any amount in the inverted vial of the brilliant green lactose bile (2%) broth fermentation tube at any time within 48 hrs. constitute a positive confirmed test.

Test Result:

Use MPN table for number of coliform bacteria present per 100 ml of water sample.

ENUMERATION OF FECAL COLIFORM

Fecal: Coliform MPN Procedure

The fecal coliform test when executed as described may be expected to differentiate between coliform of fecal origin (intestine of warm-blooded animals) and coliform from other source.

Procedure:

Select tubes of LTB that is positive for gas production. Inoculate a loop full of broth from each positive culture into a tube of Brilliant Green Lactose Bile (2%) Broth (BGLBB) and a tube of Tryptone Broth (TB)

Incubated tubes at 44 °C ± 1 °C. Observe Brilliant Green Lactose Bile (2%) Broth after 24 hrs. and 48 hrs. of incubation of gas production in the inverted Durhams tubes.

After 24 hrs. incubation, pipette aseptically 5 ml portion of each Tryptone Broth to a separate tube and test for the presence of indole as follows.

Indole Test (Kovac`s, 1928)

a. Add 0.2-0.3 ml of indole reagent to each tube and shake
b. Level tubes to stand for 10 minutes and observe result. A dark red color in the amyl acetate layer constitutes a positive reaction. An orange color indicates the probable presence of shatole sand may be reported as a ± reaction.

Result:

Cultures production gas in the presence of bile and indole at 44 °C, are presumed positive for fecal coliform organisms.

Determine the MPN of fecal coliform organisms per 100 ml of water sample.

Reference: ISO 9308-2: 1990 (E)

MOST PROBABLE NUMBER OF COLIFORM ORGANISM IN 100 ML OF THE ORIGINAL WATER

MOST PROBABLE NUMBER OF COLIFORM

Numbers of tubes	50 ml	10 ml	1 ml	Most probable number of coliform organisms in 100 ml of the original water
Number of tubes giving positive reaction	0	0	0	0
	0	0	1	1
	0	0	2	2
	0	1	0	1
	0	1	1	2
	0	1	2	3
	0	2	0	2
	0	2	1	3
	0	2	2	4
	0	3	0	3
	0	3	1	5
	0	4	0	5
	1	0	0	1
	1	0	1	3
	1	0	2	4
	1	0	3	6
	1	1	0	3
	1	1	1	5
	1	1	2	7
	1	1	3	9
	1	2	0	5
	1	2	1	7
	1	2	2	10
	1	2	3	12
	1	3	0	8
	1	3	1	11
	1	3	2	14
	1	3	3	18
	1	3	4	20
	1	4	0	13
	1	4	1	17
	1	4	2	20

Reference: Microbiological Analysis FAO

LABORATORY MANUAL

SWAB TEST

SWAB:

General:

It is important that the laboratory receive a sample which is representative of the surface tested has not been changed during transport and storage by residues disinfectants.

Disinfectants are generally formulated for disinfections contacts time of 5 minutes to investigation the surface with with swabs with to assess the performance of the cleaning and disinfection program.

An appropriate neutralize for all situation can be prescribed.

However, the components of a neutralize which may be used in most situations is given in Table-1

Collection of Sample:

Sample may be collected from the following sources:

- ✓ Table
- ✓ Utensils
- ✓ Freezing tray or Panning Tray
- ✓ Conveyer Belts (IQF)
- ✓ Workers Hand

The above sample should be drawn eithers before commencement of the work or after normal cleaning.

Preparation of Swab:

- ✓ A swab usually consists of a piece of aluminum or iron wire, 15 gauge and 15 cm long. Wooden piece of the above size specification can be also be used.
- ✓ Make one end of this rough for about 1.25 cm
- ✓ Around this rough end, roll tightly 1 gm of absorbent cotton of surgical grade, so as to make a swab of about 1.9 cm thick and 2.5 cm long.
- ✓ Place the wire with the swab in a narrow. Thick-walled test tube of 18×150 mm size.
- ✓ Plug the end of the test tube with non-absorbent cotton.
- ✓ Sterilize in the autoclave for 15 lbs. for 20 minutes.

Collection of Swab:

-The standard is for swabbing is 25 cm^2 (5 cm × 5 cm).

-Although the area for swabbing from 20 to 100 sq. cm also used as required.

- ✓ Use a steel template of correct size, which is readily sterilized by alcohol flaming to outline the area.

Procedure:

- ✓ Place the sterilized template on the surface from where the sample to be collected.
- ✓ Aseptically remove a swab from the sterile wrapping / container.
- ✓ Moistened the tip by immersing it in a tube containing dilution liquid.
- ✓ Press the tip of the swab against the wall of the tube to remove excess liquid.

- ✓ Place the tip of the swab on the surface to be investigated and streak an area of 20 cm^2 to 100 cm^2 (as per size used of the template), whilst rotating the swab between thumb and forefinger in two directions at right angles to each other.
- ✓ Put the swab in the tube with dilution liquid and aseptically break or cut off the stick.

Transport:

Transport the sample obtained with of the swab method, properly within 4 hrs. to icebox set at 1 °C to 4 °C.

Example in the laboratory as soon as possible and not late than 24 hrs.

Mixing:

Thoroughly mix the continent of the tubes containing swabs using a mixer for 30 s, adjusting the speed so that, the wall of the tube is wetted up to a height of 2 cm to 3 cm below the top.

If mixer not available, vigorously shake the contents of the tubes for about 200 time until the swab is disseminated as much as possible.

Dilutions:

Treat this initial suspension as a 1:1 dilution.

If a high numbers of microorganism as expected, prepare further decimal dilution in peptone water (0.1%) dilution to obtain countable numbers of colonies.

Aerobic Plate Count:

APC analysis: Pour 10 ml of the required dilution in to the sterile petri dishes of (100 ml mm Dia) in duplicate

- Add 20 ml of melted Plate Count Agar (PCA) at about 45 °C to the inoculated plate.
- Mix the medium and the sample by tilting and rotating the dish.
- The time limit between inoculation and addition of medium should not be exceeding 15 min.
- After solidification of the agar, invert the plates and incubate at 37 °C ± 1 °C for 48 hrs.

Calculation:

Record the numbers of colony forming unit (CFU) per milliliter of initial suspension or of its dilution(S)

The numbers of colony forming unit (CFU) per square cm of the surface investigate may be calculate by using the formula-

$$Ns = \frac{N \times F}{A}$$

Where:

N is the number of CFU in 1 ml of dilution liquid (or neutralizing fluid)

F is the amount in milliliters, of dilution fluid in the tube

A is the surface investigated, in square centimeters

Report:

Report the numbers of colony- forming unit (CFU) per square cm of the report the same along with and time of incubation.

Apparatus and glassware:

Usual microbiological laboratory apparatus, glassware and in particular of the following.

Swab:

A piece of aluminum, iron wire or wooden stick, 15 gauge and 15 cm long with a cotton swab of about 1.9 cm thick and 2.5 cm long on one end.

Template:

Made of stainless steel enclosed an area of 20 cm^2 to 100 cm^2 which is easy to clean and can be sterilized.

Mixer:

For mixing liquid in culture tubes.

Icebox:

Insulated capable of maintaining the sample at low temperature (0 to 4 °C) during transportation to the laboratory.

Containers:

Such as bottles, tubes or flasks suitable for sterilized and storage of culture media.

Required Media:

- Plate Count Agar.
- Dilution: 0.1% peptone water (0.1% Peptone water distilled water). Dehydrated Culture media is commercially available. Prepared as per manufacture' s instruction.

Reference:

Good Laboratory Practices by Samsuddin Ilias.

APPENDIX-B

(Standard of Microbiological Parameter for potable water & Ice)

Sl. No.	Parameter	Sample quantity (ml)	Guide Level (GL)	Maximum Allowable concentration
01	Total Coliform	100/ml		MPN <1
02	Fecal Coliform	100/ml		MPN <1
03	Total Bacteria (SPC) at 37 °C / at 22 °C	10	10	

FISH AND FISH PRODUCT (INSPECTION AND QUALITY CONTROL) RULES, 1997 GOVERNMENT OF BANGLADESH.

RESULT REPORTING AND RECORD KEEPING

a. Records are to be kept documenting the care and disposition of samples during their time in the laboratory. These records must show the storage condition, Personnel with custody and final description of samples when they are no longer needed.
b. Records are to be kept reading sample analysis, including sample description, storage condition sample retention, description of analytical method, all raw data, and observations, calculations and conclusion. The analyst (s) responsible for each segment of a procedure should be identified in the record.
c. The laboratories are developing their own worksheets for capturing data and result. The records are to be in the form of worksheet that become a part of the entire record for each sample or notebooks that cab is referred to in the sample record or in correspondence.
d. The analytical record is to be reviewed periodically for competences and accuracy before the result are reported
e. The in-house review of results prior to reporting are to be their step process. The results and supporting data must first be reviewed by another analyst in the laboratory other than the must one that actually prepared the data for reporting. The microbiologist and quality control officer of their designee must perform the second and their review.
f. Records are the kept of all quality assurance and quality control officer.
g. Records are to be kept on annalistically worksheet or on separate logbook that are entered as a part of the analytical routine in the laboratory.
h. The analyst responsible for each Q. A. check should be indicate and the steps taken to being into control any procedures of function, out of compliance or tolerance are to be recorded.

The system in place for storage and retrieval of analytical data of samples and quality assurance must be provided ready retrievals of all the data of generated in the laboratory upon request if necessary. The length of all time that data and others records can be kept may vary. Therefore, it is very important that the Deputy Director be notified before any and all records are removed or destroyed as specified in SOP.

SAMPLING AND TESTING RECORD:

Microbiological tests are done on every require point the process line. Usually, sample are taken at random from various point of process line given special emphasize on raw materials receive and frozen products. ICMSF (1986) testing procedure is following in factory laboratory. All kind advises are taken in this regard from the FIQC, GoB officer.

RECORD KEEPING PROCEDURE

It is very much clear that accurate documentation & keeping is an essential part of successful laboratory activities. Various recorded and monitoring documents procedure by the inspector or lab person are the main evidence of the HACCP based quality control system. All the records would be kept together and verification and filed up according to date. Those records are kept in laboratory for at least two years from the date of analysis by the company.

LABORATORY REGISTER & DOCUMENTS

SL NO	NAME
01	Sample collection register (Water & Ice)
02	Sample collection register (Swab)
03	Bacteriological Analysis Register (Final Products)
04	Bacteriological Analysis Register (Randomly)
05	Water & Ice Test Register
06	Swab Test Register
07	Media Preparation Register
08	Bacteriological Analysis Report (Raw Products)
09	Bacteriological Analysis Report (Finished Products)
10	Bacteriological Analysis Report (Random Pro.)
11	Water & Ice Test Report
12	Swab Test Report
13	Formalin Test Report
14	Lab Cleaning Register
15	Lab Equipment Calibration Records
16	Autoclave Register

GOOD LABORATORY PRACTICE (GLP)

General Cleaning and Precautions: The following minimum safety precautions should be taken at all times in a laboratory.

1. Care shall be exercised to ensure aseptic conditions and precautions are taken during handling of cultures.
2. All used materials should be sterilized
3. Immediately after use all pipettes should be immersed overnight in hypochlorite solution containing at least 500 ppm of available chlorine or any other suitable germicidal solution.
4. Benches should be swabbed before & after use with and active germicidal solution.
5. Extreme care should be taken to prevent spillage of cultures during transfer or flaming the loop. If this occurs the whole area should be treated immediately by soaking with and active germicidal solution for at least 5 minutes.
6. Always use approved protective clothing in the laboratory.
7. Never eat or drink or smoke in the laboratory or pipettes culture with the month.
8. All laboratory clothing and others items are made sterile before washing Lab-staff may remove aprons whenever they go outside the laboratory.
9. When in doubt ask an expert.

Cleaning of new glass ware:

All new glass ware shall be subjected before use to the following procedure:
i. Wash thoroughly in a hot solution of a suitable non-ionic detergent. Rinse in running tap water.

ii. Submerge the glassware for at least 16 hrs. in 1% (v/v) solution of HCL acid.

iii. After removal from acid, rinse in tap water and then distilled water.

iv. Fill with or immerse in distilled water and autoclave at 180 °C for 15 minutes

Cleaning of used laboratory:
After use, all contamination equipment together with any contents shall be sterilized by autoclaving at 15 lbs. For 30 minutes before cleaning discarding.

Test Tubes, Petri Petri dishes, Flasks and Bottles:

After sterilization discard any contents and clean as follows:

i. Rinse in warm tap water

ii. Wash in a suitable non-ionic detergent solution (Chlorine acid solution) and

brush in this solution

iii. Rinse at least six times in running water and once in distilled water. Then air dry.

Pipettes:

After disinfection, clean glass pipettes as follow:
a. Remove cotton plugs.
b. Wash the pipettes using a suitable non-ionic detergent
c. Rinse the pipettes in tap water and then in distilled water.
d. Then air dry.

Notes:

Potassium dichromate 90 gm

Water 200 ml

Sulfuric Acid (Cone) 2 Liter

Sterilization:

All equipment shall be sterilized before use. Before sterilization, pipettes, test tubes flasks and bottles shall be plugged with non absorbent cotton or protected by means of a suitable enclosure. Any piece of equipment whose external surface has to be kept sterile shall be wrapped in Kraft paper or others suitable materials.

Autoclaves:

The equipment shall be autoclaved at 121 °C for 15 minutes. The following precautions shall be observed here.

i. Cover plugs of cotton wool with Kraft paper of others suitable materials.

ii. Lessen cap closures before autoclaving and tighten again on cooling.

iii. Distribute the items within the autoclaves so that steam will be able to circulate

freely.

iv. Make sure that there is enough water in the autoclave that the coil is fully

immersed in water.

v. Ensure that all air expelled from the autoclave before it is closed.

LABORATORY PRACTICES

Introduction:

The laboratory worker is more exposed to potentially lethal hazard than an average citizen. His work involves exposure to toxic chemical, vapors, carcinogens, high voltage and injurious factors to which in the filed of microbiology there are added infections. The infected person himself can be a hazard. He can transmit infection to others. The principal hazard in a microbiological laboratory is the aerosol formation.

I) Aerosol Can am formed by:

i. Atomization of liquid suspension
ii. Light Weighted powders, infected skin scales, textile, dust.

II) Laboratory routine causing contamination:

a. Pipetting: All type of pipetting is hazard causing formation of aerosol. Hence toxic materials should be never be pipetting orally.
b. Platinum loops, scissor, plugs, and swab test: Dissemination of organisms by sputtering on flaming with contaminated loop is a potential hazard Simple withdrawal of a loop from a culture can draw out fine drops of liquid, which break up into small drops and contaminate the neck of the flasks or tubes.
c. Agar Culture: Plates, after observation, if fallen on floor cause potential source of aerosols. When laboratory personnel stoop down to pick them, they are exposed to more risk. The moisture condensing on the medium, falling down as drops may also cause danger. Mites and other insects invade the culture plates and they come out migrating from one plate to anther causing infection.

Ill) Safety measure to be adopted:

a) "Every man is his own safety officer in a laboratory" To achieve safety and reliable result, structural simplicity is quite essential, which will facilitate easy cleaning and less contamination
b) A high amount of personnel hygiene is required:

✓ As soon as possible all sickness and injuries shall be reported
✓ Unknown materials shall be handled in a similar way as that of potential pathogens
✓ The laboratory shall be clean always
✓ Ensure that, contamination apparatus culture, laboratory, clothing etc. are sterilization before they are washed up.
✓ A ready for use supply of disinfectant shall be kept.
✓ Operational technique for achieving reliable test results and safety in the laboratory.

- ✓ Overcrowding of work-benches with glass wares and others items should be avoided.
- ✓ Loop stem should be short's
- ✓ Used pipettes, plug etc. should be placed vertically in a disinfectant
- ✓ The laboratory atmosphere shall be fumigated frequently using a mixture of formalin and water.
- ✓ Entire laboratory shall be wiped off with a piece of cloth dipped in disinfectant to clear off the dust
- ✓ Laboratory conditions shall be investigated and monitored to check the sterility and effectiveness to sanitation

PROCEDURE FOR LABORATORY STORE

Requirement

Requirement shall be documented in a standard format indicating there in the name of the chemical/ appliances brand name, Quality, Rate contact, available on stock and time of delivery.

Reception and storage:

- ✓ All materials for laboratory stores, on its arrivals shall be received in stores and counter checked with reference to the order placed for quantity, brand name, date of expiry.
- ✓ All instruction indicated on the label of the primary pack/containers shall be monitored and documented.
- ✓ Storage of chemical shall be arranged in a systematic way as per the description indicated in the label oof the containers.
- ✓ Storage and inventory techniques shall be followed.
- ✓ Hazardous/toxic chemical shall be under stored safe custody

Issue and Disposal:

Chemical, media if any, which has crossed the expiry date shall be segregated and disposal off immediately to ensure it is not put into use.

Disposal of Wastage:

a) When small volume of culture alone is sterilized, all cultures will be sterilized by autoclaving 15 minutes at 121 °C prior to disposal. If cultures are sterilized along with others waste shall be sterilized one hour at 121 °C prior to disposal.
b) Large volumes of pre-enrichment cultures will be sterilized by autoclaving one hours at 121 °C prior to disposal or by autoclaving for a specific time, temperature and pressure to effectuate kill of cultures as determined by documented laboratory evaluation. For waste items held longer than 24 hrs. (in cause where the autoclave is not working or excessive amounts of waste have accumulated) they shall be treated with an appropriate disinfectant.
c) All microscope slides will be placed in an autoclavable bag or container immediately after use. This bag or container is to be sterilized by autoclaving one hour at 121 °C prior to disposal. It is recommended that regular microscope slide not be saved. Saved only especially glass slides such as those used for direct microscope, clump count, hanging drop slides or others specially slides.
d) All used disposal pipettes, Petri dishes, disposal loops, disposable needles etc. used in testing RMS samples will be placed autoclavable bags, or containers with disinfectants immediately after use. These materials will then be sterilized for 60 minutes at 121 °C prior to disposal.
e) All cultures and items to be sterilized prior to disposal are to be held in a designated area and sterilized on a daily basis. In cases where autoclavable bags or containers are not completely full and ready to be sterilized, these bags should be securely closed for overnight storage. Autoclaves bags with small amounts of waste may be reopened and re-used until filled, then sterilized.

f) Autoclaves will have periodic spore tests run during disposal of waste to check their performance. These quality assurance checks will be documented monthly in the laboratory`s quality assurance logbooks. QA checks shall be done to check the performance of the disinfected used to destroy cultures or disinfects waste products.

Laboratory Sanitation:

1. Work surfaces in the laboratory will be sanitized prior to and after use with an appropriate sanitizer. An appropriate is, so that is approved by the codex Alimentarius for this purpose.
2. Water baths will be examined weekly for cleanliness or more frequently if needed.
3. The interior and exterior of incubators will be cleaned at least quarterly or more often, if necessary, with appropriate disinfectant.
4. Compound that may be enhanced the growths of certain organism on workbench and other surface such as some phenolic compounds will be not used in the laboratory.
5. Swab tests and others appropriate tests of work surfaces balanced refrigerators, freezers, storage areas for gloves, forceps and others equipment`s used in sample preparation area will be conducted weekly to determine effectiveness of sanitation and cleaning.
6. Setting air plates will be used on a monthly basis to check air quality in the laboratory.

Personal Protection:

1. Laboratory coveralls, growths or uniforms must be worn at all times for works in the laboratory.
2. Appropriate gloves must be worn for all procedure that may involve director or accidental contact with blood, body fluids and others potentially infections materials or infected animals. After use, gloves should be removed aseptically and hands must then be washed.
3. Personnel must wash their hands after handling infectious materials and animals, and before they leave the laboratory working areas.
4. Safety glasses, face shields (visors) or others protection devices must be worn when, it is necessary to protect the eyes and face from splashes impacting objects and sources of artificial ultraviolet radiation.
5. It is prohibited to ware protective laboratory clothing outside the laboratory, e.g. in canteens, coffee rooms, offices, libraries, staff rooms, staff rooms, and toilets.
6. Open toed footwear must nit is worn in laboratories.
7. Eating, drinking, smoking, applying cosmetics and handling lenses is prohibited in the laboratory working areas.
8. Storing human foods or drinks anywhere in the laboratory working areas is prohibited.
9. Protective laboratory clothing that has been laboratory used in the laboratory must not be stored in the same lockers or cupboards as street clothing.

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